

METHODS FOR INHIBITING PROTEASOME

RELATED APPLICATIONS

This application claims priority benefit under Title 35 § 119(e) of United
5 States provisional Application No. 60/442,182, filed January 23, 2003, the contents of
which are herein incorporated by reference.

FIELD OF THE INVENTION

The present invention relates generally to a novel class of lactams, which are
10 useful as threonine protease inhibitors, and more particularly as proteasome inhibitors.
This invention also relates to pharmaceutical compositions comprising these
compounds and methods of using the same.

BACKGROUND OF THE INVENTION

15 The proteasome, also referred to as the multicatalytic proteinase complex, is
an unusually high molecular weight complex (about 700 kDa, 26S) that is found in
both the cytoplasm and nucleus of a wide variety of eukaryotic cell types. The
proteasome is comprised of the 20S central catalytic core and two 19S regulatory
caps. The 19S regulatory caps are found at each end of the 20S barrel-shaped complex
20 and regulate the entry of substrates into the central catalytic core. The 19S caps play a
role in the recognition of substrates that have been targeted for degradation by the
addition of multiple molecules of the 8.5 kDa polypeptide ubiquitin (reviewed in
Coux, O., Tanaka, K and Goldberg, A. 1996 Ann. Rev. Biochem. 65, 801-847). After
facilitating the removal of the ubiquitin molecules from the substrate, the 19S cap
25 promotes the unfolding of the substrate protein as it enters the central catalytic core.
The proteasome is highly conserved evolutionarily, having been found in all
eukaryotic cells studied, and may constitute up to 1.0% of the total protein in tissue
homogenates. The proteasome has been found in both the cytoplasm and nucleus of
cells, suggesting a functional role in both of these compartments (Tanaka et al., J. Cell
30 Physiol. 139:34-41 (1989); Amsterdam et al., Proc. Natl. Acad. Sci. USA 90:99-103
(1993).



23914

PATENT TRADEMARK OFFICE

Early studies on the proteasome lead to the delineation of five different proteolytic activities, each associated with a distinct component of the complex (Wilk and Orłowski, J. Neurochem. 35:1172-1182 (1980); Wilk and Orłowski, J. Neurochem. 40:842-849 (1983); Orłowski and Wilk, Biochem. Biophys. Res. Comm. 101:814-822 (1981)). The three major activities are similar in specificity to chymotrypsin, pepsin and peptidylglutamyl peptidase. The two other activities described exhibit a preference for cleavage of peptide bonds on the carboxyl side of branched chain amino acids and toward peptide bonds between short chain neutral amino acids (Orłowski, M. 1990 Biochemistry 29. 10289-10297).

It is now well established that the proteasome is a major extralysosomal proteolytic system involved in the proteolytic pathways essential for diverse cellular functions such as cell division, antigen processing and the degradation of short-lived regulatory proteins such as oncoproteins, transcription factors and cyclins (Ciechanover, A. (1994) Cell 79, 13-21; Palombelli, V.J., Rando, O.J., Goldberg, A.L. and Maniatis, T. (1994) Cell 78, 773-785). Since the proteasome plays a key role in the orderly degradation of cyclins during the progression of the cell cycle, it plays a role in cell division. Additional studies demonstrated that disruption in any one of 12 out of 13 genes encoding yeast proteasome subunits results in an arrest in cellular proliferation or an inability to degrade proteins, also suggesting a role for the proteasome in cell growth (Fujiwara et al, J. Biol. Chem. 265:16604-16613 (1990); Beynon, Int. Committee on Proteolysis News Letter, January, 1-2 (1994)). Therefore inhibition of the proteasome may be useful in the treatment of diseases resulting from aberrant cell division.

The observation that ubiquitin-mediated proteasomal proteolysis plays a critical role in the activation of NFκB could be exploited clinically by the use of inhibitors directed toward the proteasome. Formation of the active form of NFκB requires proteasome-mediated proteolysis of p105, the inactive precursor of NFκB. In addition, the processed form of NFκB (p65/p50) is maintained in the cytosol as an inactive complex bound to the inhibitory protein IκB. Various stimuli activate NFκB by initiating the signaling pathway which leads to the proteasome-mediated degradation of IκB. Abnormal activation of NFκB followed by the stimulation of cytokine synthesis has been observed in a variety of inflammatory and infectious

diseases. Activation of NFkB is also essential for angiogenesis and for expression of adhesion molecules, and therefore proteasome inhibitors may also have utility in the treatment of diseases associated with the vascular system.

5

SUMMARY OF THE INVENTION

The present invention is directed to methods of inhibiting proteasome which comprise administering to mammals in need thereof a therapeutically effective amount of a compound of the present invention.

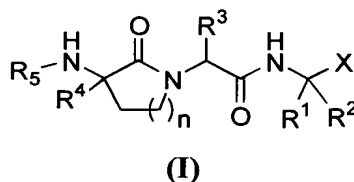
10 The present invention is also directed to methods for treating cancer which comprise administering to a mammal in need of such treatment a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

The present invention is also directed to methods for treating immunological or inflammatory conditions which comprises administering to a host in need of such treatment a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

15 The present invention is further directed to pharmaceutical compositions having proteasome inhibiting activity comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

20 These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that compounds of formula (I):

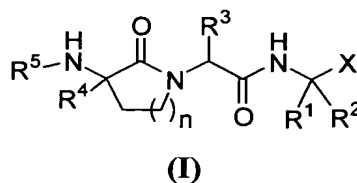
25



wherein n, R¹, R², R³, R⁴, R⁵, and X are defined below, stereoisomeric forms, mixtures of stereoisomeric forms, or pharmaceutically acceptable salt forms thereof, are effective proteasome inhibitors.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Thus, in a first embodiment, the present invention provides methods for treating cancer comprising administering to a mammal in need thereof, either alone or
 5 in combination with at least one other anticancer agent, a therapeutically effective amount of a compound of Formula I:



10

or a stereoisomer or pharmaceutically acceptable salt form thereof, wherein:

the lactam ring of Formula (I) is substituted with 0–2 R^b;

15 X is selected from the group:

B(OH)₂, BY¹Y², and C(=O)C(=O)NHR^{1a};

Y¹ and Y² are independently selected from:

- a) -OH,
 - 20 b) -F,
 - c) -NR¹⁸R¹⁹,
 - d) C₁-C₈ alkoxy, or
- when taken together, Y¹ and Y² form:
- e) a cyclic boron ester comprising from 2 to 20 carbon atoms, and,
 25 optionally, 1, 2, or 3 heteroatoms which can be N, S, or O;
 - f) a cyclic boron amide comprising from 2 to 20 carbon atoms and,
 optionally, 1, 2, or 3 heteroatoms which can be N, S, or O; or
 - g) a cyclic boron amide-ester comprising from 2 to 20 carbon atoms and,
 30 optionally, 1, 2, or 3 heteroatoms which can be N, S, or O;

R¹ is selected from the group:

- C₁₋₁₀ alkyl substituted with 0-3 R^a;
- C₂₋₁₀ alkenyl substituted with 0-3 R^a;
- C₂₋₁₀ alkynyl substituted with 0-3 R^a; and
- 5 C₃₋₆ cycloalkyl substituted with 0-3 R^a;

R^{1a} is selected from the group:

- C₁₋₁₀ alkyl substituted with 0-3 R^a;
- C₂₋₁₀ alkenyl substituted with 0-3 R^a;
- 10 C₂₋₁₀ alkynyl substituted with 0-3 R^a; and
- C₃₋₆ cycloalkyl substituted with 0-3 R^a;

R^a is selected at each occurrence from the group:

- C₁₋₃ alkyl, C₃₋₆ cycloalkyl, Cl, F, Br, I, CF₃, OH, =O, C₁₋₆ alkoxy, SH,
- 15 -S-C₁₋₆ alkyl;
- phenyl substituted with 0-3 R^b;
- naphthyl substituted with 0-3 R^b;
- O-(CH₂)_q-phenyl substituted with 0-3 R^b;
- O-(CH₂)_q-naphthyl substituted with 0-3 R^b; and
- 20 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group:
O, S, and N, and substituted with 0-3 R^b;

R^b is selected at each occurrence from the group:

- 25 C₁₋₆ alkyl, Cl, F, Br, I, OH, C₁₋₆ alkoxy, -CN, -NO₂, C(O)OR⁷, NR^dR^d, CF₃,
OCF₃, and C₃₋₆ cycloalkyl;

R² is H;

- 30 alternatively, R¹ and R² combine to form a C₃₋₅ cycloalkyl group;

R³ is selected from the group:

- C₁₋₆ alkyl substituted with 0-2 R^a;
- C₂₋₆ alkenyl substituted with 0-2 R^a;
- 5 C₂₋₆ alkynyl substituted with 0-2 R^a;
- (CH₂)_q-C₃₋₆ cycloalkyl substituted with 0-2 R^a;
- (CH₂)_q-phenyl substituted with 0-2 R^a;
- (CH₂)_q-naphthyl substituted with 0-2 R^a; and
- (CH₂)_q-5-10 membered heteroaryl consisting of carbon atoms and 1-4
- 10 heteroatoms selected from the group:
O, S, and N, and substituted with 0-2 R^a;

R⁴ is selected from the group:

- H;
- 15 C₁₋₆ alkyl substituted with 0-3 R^b;
- phenyl substituted with 0-3 R^b;
- benzyl substituted with 0-3 R^b; and
- phenethyl substituted with 0-3 R^b;

20 R⁵ is H or Q-R^{5a};

Q is 0, 1, 2, or 3 amino acids;

R^{5a} is selected from the group:

- 25 -S(O)R⁶, -S(O)₂R⁶, -C(O)R⁶, -C(O)OR⁸, -C(O)NHR⁶, C₁₋₃ alkyl-R^{6a}, C₂₋₆
- alkenyl-R^{6a}, and C₂₋₆ alkynyl-R^{6a};

R⁶ is selected from the group:

- C₁₋₆ alkyl substituted with 0-3 R^c;
- 30 phenyl substituted with 0-3 R^c;

naphthyl substituted with 0-3 R^c;
benzyl substituted with 0-3 R^c; and
5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group:

5 O, S, and N, substituted with 0-3 R^c;

R^{6a} is selected from the group:

phenyl substituted with 0-3 R^c;
naphthyl substituted with 0-3 R^c;
10 benzyl substituted with 0-3 R^c; and
5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group:
O, S, and N, substituted with 0-3 R^c;

15 R^c is selected at each occurrence from the group:

C₁₋₄ alkyl, C₁₋₄ alkoxy, CF₃, OCF₃, Cl, F, Br, I, =O, OH, phenyl, C(O)OR⁷,
NR^dR^d, -CN, and NO₂;

R^d is selected at each occurrence from the group:

20 H and CH₃;

R⁷ is selected at each occurrence from the group:

H and C₁₋₆ alkyl;

25 R⁸ is selected from the group:

C₁₋₆ alkyl, benzyl, and C₃₋₆ cycloalkyl-methyl;

R¹⁸ and R¹⁹ at each occurrence are independently selected from H, C₁₋₄ alkyl,
aryl(C₁₋₄ alkyl)-, and C₃₋₇ cycloalkyl;

30

n is selected from the group:

1, 2, and 3; and

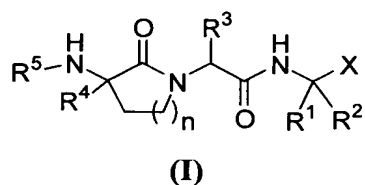
q is selected from the group:

0, 1, and 2.

5

In a preferred embodiment, the present invention provides methods for treating cancer comprising administering to a mammal in need thereof, either alone or in combination with at least one other anticancer agent, a compound having Formula (I):

10



or a stereoisomer or pharmaceutically acceptable salt form thereof, wherein:

15

the lactam ring of Formula (I) is substituted with 0-2 R^b;

X is selected from the group:

B(OH)₂, BY¹Y², and C(=O)C(=O)NHR^{1a};

20

Y¹ and Y² are independently selected from:

- a) -OH,
- b) -F,
- c) -NR¹⁸R¹⁹,
- d) C₁-C₈ alkoxy, or

25

when taken together, Y¹ and Y² form:

- e) a cyclic boron ester comprising from 2 to 20 carbon atoms, and, optionally, 1, 2, or 3 heteroatoms which can be N, S, or O;
- f) a cyclic boron amide comprising from 2 to 20 carbon atoms and, optionally, 1, 2, or 3 heteroatoms which can be N, S, or O; or

30

- g) a cyclic boron amide-ester comprising from 2 to 20 carbon atoms and, optionally, 1, 2, or 3 heteroatoms which can be N, S, or O;

R¹ is selected from the group:

- 5 C₁₋₆ alkyl substituted with 0-3 R^a;
 C₂₋₆ alkenyl substituted with 0-3 R^a;
 C₂₋₆ alkynyl substituted with 0-3 R^a; and
 C₃₋₆ cycloalkyl substituted with 0-3 R^a;

10 R^{1a} is selected from the group:

- C₁₋₁₀ alkyl substituted with 0-3 R^a;
 C₂₋₁₀ alkenyl substituted with 0-3 R^a;
 C₂₋₁₀ alkynyl substituted with 0-3 R^a; and
 C₃₋₆ cycloalkyl substituted with 0-3 R^a;

15

R^a is selected at each occurrence from the group:

- C₁₋₃ alkyl, C₃₋₆ cycloalkyl, Cl, F, Br, I, CF₃, OH, =O, C₁₋₆ alkoxy, SH,
 -S-C₁₋₆ alkyl;

phenyl substituted with 0-3 R^b;

20 naphthyl substituted with 0-3 R^b;

-O-(CH₂)_q-phenyl substituted with 0-3 R^b;

-O-(CH₂)_q-naphthyl substituted with 0-3 R^b; and

5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
 selected from the group:

25 O, S, and N, and substituted with 0-3 R^b;

R^b is selected at each occurrence from the group:

- C₁₋₆ alkyl, Cl, F, Br, I, OH, C₁₋₆ alkoxy, -CN, -NO₂, C(O)OR⁷, NR^dR^d, CF₃,
 OCF₃, and C₃₋₆ cycloalkyl;

30

R² is H;

alternatively, R¹ and R² combine to form a C₃₋₅ cycloalkyl group;

5 R³ is selected from the group:

C₁₋₆ alkyl substituted with 0-2 R^a;

C₂₋₆ alkenyl substituted with 0-2 R^a;

C₂₋₆ alkynyl substituted with 0-2 R^a;

-(CH₂)_q-C₃₋₆ cycloalkyl substituted with 0-2 R^a;

10 -(CH₂)_q-phenyl substituted with 0-2 R^a;

-(CH₂)_q-naphthyl substituted with 0-2 R^a; and

-(CH₂)_q-5-10 membered heteroaryl consisting of carbon atoms and 1-4

heteroatoms selected from the group:

O, S, and N, and substituted with 0-2 R^a;

15

R⁴ is selected from the group:

H;

C₁₋₆ alkyl substituted with 0-3 R^b;

phenyl substituted with 0-3 R^b;

20 benzyl substituted with 0-3 R^b; and

phenethyl substituted with 0-3 R^b;

R⁵ is H or Q-R^{5a};

25 Q is 0, 1, 2, or 3 amino acids;

R^{5a} is selected from the group:

-S(O)R⁶, -S(O)₂R⁶, -C(O)R⁶, -C(O)OR⁸, -C(O)NHR⁶, C₁₋₃ alkyl-R^{6a}, C₂₋₆
alkenyl-R^{6a}, and C₂₋₆ alkynyl-R^{6a};

30

R⁶ is selected from the group:

- C₁₋₆ alkyl substituted with 0-3 R^c;
- phenyl substituted with 0-3 R^c;
- naphthyl substituted with 0-3 R^c;
- 5 benzyl substituted with 0-3 R^c; and
- 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group:
O, S, and N, substituted with 0-3 R^c;

10 R^{6a} is selected from the group:

- phenyl substituted with 0-3 R^c;
- naphthyl substituted with 0-3 R^c;
- benzyl substituted with 0-3 R^c; and
- 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
15 selected from the group:
O, S, and N, substituted with 0-3 R^c;

R^c is selected at each occurrence from the group:

- C₁₋₄ alkyl, C₁₋₄ alkoxy, CF₃, OCF₃, Cl, F, Br, I, =O, OH, phenyl, C(O)OR⁷,
- 20 NR^dR^d, -CN, and NO₂;

R^d is selected at each occurrence from the group:

H and CH₃;

25 R⁷ is selected at each occurrence from the group:

H and C₁₋₆ alkyl;

R⁸ is selected from the group:

- C₁₋₆ alkyl, benzyl, and C₃₋₆ cycloalkyl-methyl;
- 30

R¹⁸ and R¹⁹ at each occurrence are independently selected from H, C₁-C₄ alkyl, aryl(C₁-C₄ alkyl)-, and C₃-C₇ cycloalkyl;

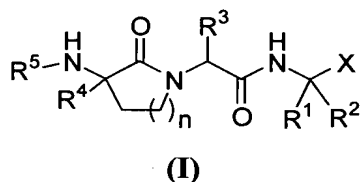
n is selected from the group:

5 1, 2, and 3; and

q is selected from the group:

0, 1, and 2.

10 In a further preferred embodiment, the present invention provides methods for treating cancer comprising administering to a mammal in need thereof a compound having Formula (I),



15

or a stereoisomer or pharmaceutically acceptable salt form thereof, wherein:

the lactam ring of Formula (I) is substituted with 0-2 R^b;

20

X is selected from the group:

B(OH)₂ and BY¹Y²;

Y¹ and Y² are independently selected from:

25

a) -OH,

b) C₁-C₈ alkoxy, or

when taken together, Y¹ and Y² form:

c) a cyclic boron ester comprising from 2 to 20 carbon atoms;

30 R¹ is selected from the group:

C₁₋₆ alkyl substituted with 0-3 halogen; and

C₂₋₆ alkenyl substituted with 0-3 halogen;

R^a is selected at each occurrence from the group:

5 C₁₋₃ alkyl, C₃₋₆ cycloalkyl, Cl, F, Br, I, CF₃, OH, =O, C₁₋₆ alkoxy, SH,
-S-C₁₋₆ alkyl;

phenyl substituted with 0-3 R^b;

naphthyl substituted with 0-3 R^b;

-O-(CH₂)_q-phenyl substituted with 0-3 R^b;

10 -O-(CH₂)_q-naphthyl substituted with 0-3 R^b; and

5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group: O, S, and N, and substituted with 0-3 R^b;

R^b is selected at each occurrence from the group:

15 C₁₋₆ alkyl, Cl, F, Br, I, OH, C₁₋₆ alkoxy, -CN, -NO₂, C(O)OR⁷, NR^dR^d, CF₃,
OCF₃, and C₃₋₆ cycloalkyl;

R² is H;

20 R³ is selected from the group:

C₁₋₆ alkyl substituted with 0-2 R^a;

C₂₋₆ alkenyl substituted with 0-2 R^a;

C₂₋₆ alkynyl substituted with 0-2 R^a;

-(CH₂)_q-C₃₋₆ cycloalkyl substituted with 0-2 R^a;

25 -(CH₂)_q-phenyl substituted with 0-2 R^a;

-(CH₂)_q-naphthyl substituted with 0-2 R^a; and

-(CH₂)_q-5-10 membered heteroaryl consisting of carbon atoms and 1-4

heteroatoms selected from the group: O, S, and N, and substituted with
0-2 R^a;

30

R⁴ is selected from the group:

- H;
- C₁₋₆ alkyl substituted with 0-3 R^b;
- phenyl substituted with 0-3 R^b;
- 5 benzyl substituted with 0-3 R^b; and
- phenethyl substituted with 0-3 R^b;

R⁵ is H or Q-R^{5a};

- 10 Q is 0, 1, 2, or 3 amino acids;

R^{5a} is selected from the group:

- S(O)R⁶, -S(O)₂R⁶, -C(O)R⁶, -C(O)OR⁸, -C(O)NHR⁶, C₁₋₃ alkyl-R^{6a}, C₂₋₆
- 15 alkenyl-R^{6a}, and C₂₋₆ alkynyl-R^{6a};

R⁶ is selected from the group:

- C₁₋₆ alkyl substituted with 0-3 R^c;
- phenyl substituted with 0-3 R^c;
- naphthyl substituted with 0-3 R^c;
- 20 benzyl substituted with 0-3 R^c; and
- 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
- selected from the group: O, S, and N, substituted with 0-3 R^c;

R^{6a} is selected from the group:

- 25 phenyl substituted with 0-3 R^c;
- naphthyl substituted with 0-3 R^c;
- benzyl substituted with 0-3 R^c; and
- 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
- selected from the group: O, S, and N, substituted with 0-3 R^c;

30

R^c is selected at each occurrence from the group:

C₁₋₄ alkyl, C₁₋₄ alkoxy, CF₃, OCF₃, Cl, F, Br, I, =O, OH, phenyl, C(O)OR⁷,
NR^dR^d, -CN, and NO₂;

R^d is selected at each occurrence from the group:

5 H and CH₃;

R⁷ is selected at each occurrence from the group:

H and C₁₋₆ alkyl;

10 R⁸ is selected from the group:

C₁₋₆ alkyl, benzyl, and C₃₋₆ cycloalkyl-methyl;

n is selected from the group:

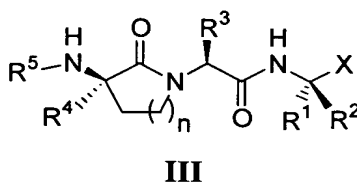
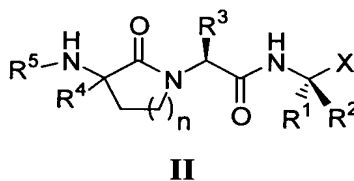
1, 2, and 3; and

15

q is selected from the group:

0, 1, and 2.

20 A further preferred embodiment of the present is directed to methods for
treating cancer comprising administering to a mammal in need thereof, either alone or
in combination with at least one other anticancer agent, a compound having Formula
II or III:



or a stereoisomer or pharmaceutically acceptable salt form thereof, wherein:

X is a boronic acid or a boron ester of formula BY^1Y^2 ;

5

Y^1 and Y^2 are independently selected from:

a) C_1 - C_6 alkoxy, or

when taken together, Y^1 and Y^2 form:

b) a cyclic boron ester comprising from 2 to 16 carbon atoms;

10

R^1 is selected from the group:

ethyl, n-propyl, i-propyl, n-butyl, allyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl,
3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and 3-butenyl;

15 R^a is selected at each occurrence from the group:

C_{1-3} alkyl, C_{3-6} cycloalkyl, Cl, F, Br, I, CF_3 , OH, =O, C_{1-6} alkoxy, SH,
-S- C_{1-6} alkyl;

phenyl substituted with 0-3 R^b ;

naphthyl substituted with 0-3 R^b ;

20 -O-(CH_2)_q-phenyl substituted with 0-3 R^b ;

-O-(CH_2)_q-naphthyl substituted with 0-3 R^b ; and

5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group: O, S, and N, and substituted with 0-3 R^b ;

25 R^b is selected at each occurrence from the group:

C_{1-6} alkyl, Cl, F, Br, I, OH, C_{1-6} alkoxy, -CN, -NO₂, C(O)OR⁷, NR^dR^d, CF₃,
OCF₃, and C_{3-6} cycloalkyl;

R^2 is H;

30

R^3 is selected from the group:

- C_{1-6} alkyl substituted with 0-2 R^a ;
 C_{2-6} alkenyl substituted with 0-2 R^a ;
 C_{2-6} alkynyl substituted with 0-2 R^a ;
 $-(CH_2)_q-C_{3-6}$ cycloalkyl substituted with 0-2 R^a ;
5 $-(CH_2)_q$ -phenyl substituted with 0-2 R^a ;
 $-(CH_2)_q$ -naphthyl substituted with 0-2 R^a ;
 $-(CH_2)_q$ -5-10 membered heteroaryl consisting of carbon atoms and 1-4
heteroatoms selected from the group: O, S, and N, and substituted with
0-2 R^a ;
10
 R^4 is selected from the group:
H, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl;
phenyl substituted with 0-3 R^b ;
benzyl substituted with 0-3 R^b ; and
15 phenethyl substituted with 0-3 R^b ;

 R^5 is H or $Q-R^{5a}$;

Q is 0, 1, or 2 amino acids;
20
 R^{5a} is selected from the group:
 $-S(O)R^6$, $-S(O)_2R^6$, $-C(O)R^6$, $-C(O)OR^8$, $-C(O)NHR^6$, C_{1-3} alkyl- R^{6a} , C_{2-6}
alkenyl- R^{6a} , and C_{2-6} alkynyl- R^{6a} ;

25 R^6 is selected from the group:
 C_{1-6} alkyl substituted with 0-3 R^c ;
phenyl substituted with 0-3 R^c ;
naphthyl substituted with 0-3 R^c ;
benzyl substituted with 0-3 R^c ; and

5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
selected from the group: O, S, and N, substituted with 0-3 R^c;

R^{6a} is selected from the group:

- 5 phenyl substituted with 0-3 R^c;
 naphthyl substituted with 0-3 R^c;
 benzyl substituted with 0-3 R^c; and
 5-10 membered heteroaryl consisting of carbon atoms and 1-4 heteroatoms
 selected from the group: O, S, and N, substituted with 0-3 R^c;

10

R^c is selected at each occurrence from the group:

C₁₋₄ alkyl, C₁₋₄ alkoxy, CF₃, OCF₃, Cl, F, Br, I, =O, OH, phenyl, C(O)OR⁷,
NR^dR^d, -CN, and NO₂;

- 15 R^d is selected at each occurrence from the group:

H and CH₃;

R⁷ is selected at each occurrence from the group:

H and C₁₋₆ alkyl;

20

R⁸ is selected from the group:

C₁₋₆ alkyl, benzyl, and C₃₋₆ cycloalkyl-methyl;

n is 1 or 2; and

25

q is selected from the group:

0, 1, and 2.

- 30 A further preferred embodiment of the present invention provides methods for
treating cancer comprising administering to a mammal in need thereof a compound

having formula III wherein X is a boronic acid or boron ester, wherein the ester is a diol selected from the group:

pinanediol, pinacol, 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, and 1,2-dicyclohexylethanediol;

R¹ is selected from the group:

ethyl, n-propyl, i-propyl, n-butyl, allyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, 3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and 3-butenyl;

R² is H;

R³ is selected from the group:

n-propyl, n-butyl, i-butyl, n-pentyl, neo-pentyl, cyclohexylmethyl, cyclopentylmethyl, phenyl, benzyl, t-butoxymethyl, benzyloxymethyl, hydroxymethyl, methoxymethyl, ethoxymethyl, propoxymethyl, and i-propoxymethyl;

R⁴ is selected from the group:

methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, phenyl, benzyl, and phenethyl;

R⁵ is H or Q-R^{5a};

Q is 0, 1, or 2 amino acids;

R^{5a} is selected from the group:

-S(O)₂R⁶, -C(O)R⁶, -C(O)OR⁸, -C(O)NHR⁶, and -CH₂-R^{6a};

R⁶ is selected from the group:

methyl substituted with 0-3 R^c;
ethyl substituted with 0-3 R^c;

propyl substituted with 0-3 R^c;
butyl substituted with 0-3 R^c;
phenyl substituted with 0-3 R^c;
naphthyl substituted with 0-3 R^c;
5 benzyl substituted with 0-3 R^c; and
quinolinyll substituted with 0-3 R^c;

R^{6a} is selected from the group:

phenyl substituted with 0-3 R^c;
10 naphthyl substituted with 0-3 R^c;
benzyl substituted with 0-3 R^c; and
quinolinyll substituted with 0-3 R^c;

R^c is selected at each occurrence from the group:

15 methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, methoxy, ethoxy,
propoxy, i-propoxy, CF₃, OCF₃, Cl, F, Br, I, OH, phenyl, C(O)OH,
NH₂, -CN, and NO₂;

R⁸ is methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, and benzyl;
20 and

n is 1 or 2.

In a further preferred embodiment of the present invention, methods are
25 provided for treating cancer, comprising administering a compound having Formula
III wherein:

X is a boronic acid or a boron ester of formula BY¹Y²;

Y¹ and Y² are individually selected from C₁-C₆ alkoxy, or when taken together, Y¹ and Y² form a cyclic boron ester where said chain or ring contains from 2 to 14 carbon atoms;

- 5 R¹ is selected from the group:
ethyl, n-propyl, i-propyl, n-butyl, i-butyl, allyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, 3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and 3-butenyl;

R² is H;

10

- R³ is selected from the group:
i-butyl, neo-pentyl, cyclohexylmethyl, t-butoxymethyl, benzyloxymethyl, hydroxymethyl, benzyl and phenyl;

- 15 R⁴ is selected from the group:
ethyl, n-propyl, i-propyl, R-2-butyl, S-2-butyl, phenyl, benzyl, and phenethyl;

R⁵ is selected from the group:

- 20 H,
benzyl,
m-methylphenylsulfonyl,
m-trifluoromethylphenylsulfonyl,
p-i-propylphenylsulfonyl,
p-propylphenylsulfonyl,
25 p-t-butylphenylsulfonyl,
p-carboxylphenylsulfonyl,
4-(1,1')biphenylsulfonyl,
1-naphthylsulfonyl,
2-naphthylsulfonyl,
30 8-quinolinylsulfonyl,
pyrazin-2-ylcarbonyl,
n-butylsulfonyl,

N-phenylaminocarbonyl,
 N-(p-n-butylphenyl)aminocarbonyl,
 benzyloxycarbonyl,
 methoxycarbonyl,
 5 t-butyloxycarbonyl,
 benzoyl,
 methanesulfonyl,
 phenylsulfonyl,
 o-nitrophenylsulfonyl,
 10 m-nitrophenylsulfonyl, and
 m-aminophenylsulfonyl; and

n is 1 or 2.

15 In a further preferred embodiment of the present invention, methods are
 provided for treating cancer comprising administering to a mammal in need thereof a
 compound having Formula III, as described above, wherein:

X is a boronic acid or boron ester, wherein the ester is a diol selected from the group:
 20 pinanediol, pinacol, 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 2,3-
 butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, and 1,2-
 dicyclohexylethanediol;

R¹ is selected from the group:
 25 ethyl, n-propyl, i-propyl, n-butyl, i-butyl, allyl, 2,2,2-trifluoroethyl, 2,2-
 difluoroethyl, 3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and 3-butenyl;

R² is H;

30 R³ is selected from the group:
 i-butyl, neo-pentyl, cyclohexylmethyl, t-butoxymethyl, benzyloxymethyl,
 hydroxymethyl, benzyl, and phenyl;

R⁴ is selected from the group:

ethyl, n-propyl, i-propyl, R-2-butyl, S-2-butyl, phenyl, benzyl, and phenethyl;

5 R⁵ is selected from the group:

H,
benzyl,
m-methylphenylsulfonyl,
m-trifluoromethylphenylsulfonyl,
10 p-i-propylphenylsulfonyl,
p-propylphenylsulfonyl,
p-t-butylphenylsulfonyl,
p-carboxylphenylsulfonyl,
4-(1,1')biphenylsulfonyl,
15 1-naphthylsulfonyl,
2-naphthylsulfonyl,
8-quinoliny sulfonyl,
pyrazin-2-ylcarbonyl,
n-butylsulfonyl,
20 N-phenylaminocarbonyl,
N-(p-n-butylphenyl)aminocarbonyl,
benzyloxycarbonyl,
methoxycarbonyl,
t-butyloxycarbonyl,
25 benzoyl,
methanesulfonyl,
phenylsulfonyl,
o-nitrophenylsulfonyl,
m-nitrophenylsulfonyl, and
30 m-aminophenylsulfonyl; and

n is 1 or 2.

In another preferred embodiment, the compound of Formula (I) is selected from the group:

- 5 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-pyrrolidinyl)propanoyl}amino)-3-butenylboronic acid (+)-pinanediol ester;
- 10 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-piperidinyl)propanoyl}amino)-3-butenylboronic acid (+)-pinanediol ester;
- 15 (1*R*)-1-(((3-((methylsulfonyl)amino)-2-oxohexahydro-1*H*-azepin-1-yl}acetyl)amino)propylboronic acid (+)-pinanediol ester;
- 20 (1*R*)-1-(((2*S*)-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)-3-cyclohexylpropanoyl)amino}propylboronic acid (+)-pinanediol ester hydrochloride;
- 25 (1*R*)-1-(((2*S*)-2-{3-(((1,1'-biphenyl)-4-ylsulfonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)amino)propylboronic acid (+)-pinanediol ester;
- 30 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)-1-pyrrolidinyl)propanoyl)amino}propylboronic acid (+)-pinanediol ester;
- 35 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((1-naphthylsulfonyl)amino)-2-oxo-1-pyrrolidinyl)propanoyl)amino)propylboronic acid (+)-pinanediol ester;
- 40 (1*R*)-1-(((2*S*)-2-{3-((anilinocarbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)amino)propylboronic acid (+)-pinanediol ester;

- (1*R*)-1-{{{(2*S*)-3-cyclohexyl-2-(3-isopropyl-3-{{{(3-methylphenyl)sulfonyl}amino}-2-oxo-1-pyrrolidinyl)propanoyl)amino}propylboronic acid (+)-pinanediol ester;
- 5 (1*R*)-1-{{{(2*S*)-3-cyclohexyl-2-(3-isopropyl-3-{{{(3-methylphenyl)sulfonyl}amino}-2-oxo-1-pyrrolidinyl)propanoyl)amino}propylboronic acid
- (1*R*)-1-{{{(3-{{{(benzyloxy)carbonyl}amino}-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 10 (1*R*)-1-{{{(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester hydrochloride;
- 15 (1*R*)-1-{{{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- (1*R*)-1-{{{(3-isopropyl-2-oxo-3-{{{(4-propylphenyl)sulfonyl}amino}-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 20 (1*R*)-1-{{{(2*S*)-2-(3-{{{(benzyloxy)carbonyl}amino}-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl)amino}propylboronic acid (+)-pinanediol ester;
- (1*R*)-1-{{{(2*S*)-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl)amino}propylboronic acid (+)-pinanediol ester
- 25 hydrochloride;
- (1*R*)-1-(((2*S*)-2-{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}-4-methylpentanoyl)amino)propylboronic acid (+)-pinanediol ester;
- 30

- (1*R*)-1-(((2*S*)-2-(3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)-1-pyrrolidinyl)-4-methylpentanoyl)amino}propylboronic acid (+)-pinanediol ester;
- 5 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-ethyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-pyrrolidinyl)propanoyl)amino)-3-butenylboronic acid (+)-pinanediol ester;
- 10 (1*R*)-1-(((2*S*)-2-(3-(((benzyloxy)carbonyl)amino)-3-isopropyl-2-oxo-1-piperidinyl)-3-cyclohexylpropanoyl)amino}propylboronic acid (+)-pinanediol ester;
- (1*R*)-1-(((3-(((tert-butoxycarbonyl)amino)-3-isopropyl-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 15 (1*R*)-1-(((3-amino-3-isopropyl-2-oxo-1-piperidinyl)(phenyl)acetyl)amino}propylboronic acid hydrochloride (+)-pinanediol ester;
- (1*R*)-1-(((3-isopropyl-3-((methoxycarbonyl)amino)-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 20 (1*R*)-1-(((3-(benzoylamino)-3-isopropyl-2-oxo-1-piperidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- (1*R*)-1-(((3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 25 and
- (1*R*)-1-(((3-isopropyl-3-(((3-methylphenyl)sulfonyl)amino)-2-oxo-1-piperidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester;
- 30

- (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-pyrrolidinyl)propanoyl}amino)-3-butenylboronic acid;
- 5 (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-piperidinyl)propanoyl}amino)-3-butenylboronic acid;
- 10 (1*R*)-1-(((3-(((methylsulfonyl)amino)-2-oxohexahydro-1*H*-azepin-1-yl}acetyl)amino)propylboronic acid (+)-;
- (1*R*)-1-(((2*S*)-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)-3-cyclohexylpropanoyl)amino}propylboronic acid;
- 15 (1*R*)-1-(((2*S*)-2-{3-(((1,1'-biphenyl)-4-ylsulfonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)amino)propylboronic acid;
- (1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)-1-pyrrolidinyl)propanoyl)amino}propylboronic acid;
- 20 acid;
- (1*R*)-1-(((2*S*)-3-cyclohexyl-2-{3-isopropyl-3-(((1-naphthylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}propanoyl)amino)propylboronic acid;
- 25 (1*R*)-1-(((2*S*)-2-{3-(((anilinocarbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)amino)propylboronic acid;
- (1*R*)-1-(((3-(((benzyloxy)carbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid;
- 30 (1*R*)-1-(((3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-hydrochloride;

- (1*R*)-1-{{{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}(phenyl)acetyl)amino}propylboronic acid;
- 5 (1*R*)-1-{{{3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)}-1-pyrrolidinyl}(phenyl)acetyl)amino}propylboronic acid;
- (1*R*)-1-{{{2*S*}-2-(3-(((benzyloxy)carbonyl)amino)}-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl}amino}propylboronic acid;
- 10 (1*R*)-1-{{{2*S*}-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl}amino}propylboronic acid hydrochloride;
- (1*R*)-1-(((2*S*)-2-{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}-4-methylpentanoyl)amino)propylboronic acid;
- 15 (1*R*)-1-{{{2*S*}-2-(3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)}-1-pyrrolidinyl)-4-methylpentanoyl}amino}propylboronic acid;
- (1*R*)-1-{{{2*S*}-3-cyclohexyl-2-(3-ethyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-pyrrolidinyl)propanoyl}amino)-3-butenylboronic acid;
- 20 (1*R*)-1-{{{2*S*}-2-(3-(((benzyloxy)carbonyl)amino)}-3-isopropyl-2-oxo-1-piperidinyl)-3-cyclohexylpropanoyl}amino}propylboronic acid;
- 25 (1*R*)-1-{{{3-(((tert-butoxycarbonyl)amino)-3-isopropyl-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid;
- (1*R*)-1-{{{3-amino-3-isopropyl-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid hydrochloride;
- 30

(1*R*)-1-{{{3-isopropyl-3-((methoxycarbonyl)amino)-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid;

5 (1*R*)-1-{{{3-(benzoylamino)-3-isopropyl-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid;

(1*R*)-1-{{{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid; and

10 (1*R*)-1-{{{3-isopropyl-3-{{{3-methylphenyl)sulfonyl}amino}-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid;

or a pharmaceutically acceptable salt form thereof.

15 In another embodiment, the present invention provides a novel pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt or solvate form thereof.

20 In another embodiment, the present invention provides methods for inhibiting proteasome in a mammal in need thereof with a therapeutically effective amount of a compound of Formula I, II or III as defined herein or a pharmaceutically acceptable salt form thereof.

25 In another embodiment, the present invention provides methods for immunomodulation in mammals in need of such treatment with a compound of Formula I, II, or III or a pharmaceutically acceptable salt form thereof.

30 In another embodiment, the present invention provides the use of compounds of Formula I, II or III or pharmaceutically acceptable salt forms thereof for the manufacture of a medicament for the treatment of cancer.

DEFINITIONS

The term "substituted", as used herein, means that any one or more hydrogens on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens on the atom are replaced. Keto substituents are not present on aromatic moieties. When a ring system (e.g., carbocyclic or heterocyclic) is said to be substituted with a carbonyl group or a double bond, it is intended that the carbonyl group or double bond be part (i.e., within) of the ring.

The present invention is intended to include all isotopes of atoms occurring in the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.

When any variable (e.g., R^a) occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R^a, then said group may optionally be substituted with up to two R^a groups and R^a at each occurrence is selected independently from the definition of R^a. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

When a bond to a substituent is shown to cross a bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring. When a substituent is listed without indicating the atom via which such substituent is bonded to the rest of the compound of a given formula, then such substituent may be bonded via any atom in such substituent. Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" or "alkylene" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. C₁₋₁₀ alkyl (or alkylene), is intended to include C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, and C₁₀ alkyl groups. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, and s-pentyl.

"Haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for example $-C_vF_w$ where $v = 1$ to 3 and $w = 1$ to $(2v+1)$). Examples of haloalkyl include, but are not limited to, trifluoromethyl, difluoroethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl. "Alkoxy" represents an alkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge. C_{1-10} alkoxy, is intended to include C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , and C_{10} alkoxy groups. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxy, and s-pentoxy. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. C_{3-6} cycloalkyl, is intended to include C_3 , C_4 , C_5 , and C_6 cycloalkyl groups. "Alkenyl" or "alkenylene" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl and propenyl. C_{2-10} alkenyl (or alkenylene), is intended to include C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , and C_{10} alkenyl groups. "Alkynyl" or "alkynylene" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl and propynyl. C_{2-10} alkynyl (or alkynylene), is intended to include C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , and C_{10} alkynyl groups.

"Halo" or "halogen" as used herein refers to fluoro, chloro, bromo, and iodo, preferably fluoro, chloro, and bromo. "Counterion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, or sulfate.

As used herein, the term "heterocycle" or "heterocyclic group" is intended to mean a stable 5, 6, or 7-membered monocyclic or bicyclic or 7, 8, 9, or 10-membered bicyclic heterocyclic ring which is saturated, partially unsaturated or unsaturated (aromatic), and which consists of carbon atoms and 1, 2, 3, or 4 heteroatoms independently selected from the group consisting of N, NH, O and S and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The nitrogen and sulfur heteroatoms may optionally be oxidized. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon

atom which results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. A nitrogen in the heterocycle may optionally be quaternized. It is preferred that when the total number of S and O atoms in the heterocycle exceeds 1, then these

5 heteroatoms are not adjacent to one another. It is preferred that the total number of S and O atoms in the heterocycle is not more than 1. As used herein, the term "aromatic heterocyclic group" or "heteroaryl" is intended to mean a stable 5, 6, or 7-membered monocyclic or bicyclic or 7, 8, 9, or 10-membered bicyclic heterocyclic aromatic ring which consists of carbon atoms and 1, 2, 3, or 4 heterotams independently selected

10 from the group consisting of N, NH, O and S. It is to be noted that total number of S and O atoms in the aromatic heterocycle is not more than 1.

Examples of heterocycles, including heteroaryls, include, but are not limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl,

15 benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4*aH*-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2*H*,6*H*-1,5,2-dithiazinyl, dihydrofuro(2,3-*b*)tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1*H*-indazolyl, indolenyl, indolinyl, indoliziny, indolyl, 3*H*-indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl,

20 isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl,

25 piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2*H*-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4*H*-quinoliziny, quinoxaliny, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6*H*-1,2,5-

30 thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl,

1,3,4-triazolyl, and xanthenyl. Preferred 5 to 10 membered heterocycles include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrazinyl, piperazinyl, imidazolyl, indolyl, benzimidazolyl, 1*H*-indazolyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, benzoxazolyl, oxindolyl, benzoxazoliny, benzthiazolyl, benzisothiazolyl, isatinoyl, isoxazolopyridinyl, isothiazolopyridinyl, thiazolopyridinyl, oxazolopyridinyl, imidazolopyridinyl, and pyrazolopyridinyl. Preferred 5 to 6 membered heterocycles include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrazinyl, piperazinyl, imidazolyl, and oxazolidinyl. Also included are fused ring and spiro compounds containing, for example, the above heterocycles. Preferred 5 to 10 membered heteroaryls include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrazinyl, imidazolyl, indolyl, benzimidazolyl, 1*H*-indazolyl, benzotriazolyl, benzisoxazolyl, benzoxazolyl, benzthiazolyl, and benzisothiazolyl. Preferred 5 to 6 membered heteroaryls include, but are not limited to, pyridinyl, furanyl, thienyl, pyrazolyl, pyrazinyl, and imidazolyl. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

The term "amino acid" as used herein means an organic compound containing both a basic amino group and an acidic carboxyl group. Included within this term are natural amino acids (e.g., L-amino acids), modified and unusual amino acids (e.g., D-amino acids), as well as amino acids which are known to occur biologically in free or combined form but usually do not occur in proteins. Included within this term are modified and unusual amino acids, such as those disclosed in, for example, Roberts and Vellaccio (1983) The Peptides, 5: 342-429, the teaching of which is hereby incorporated by reference. Natural protein occurring amino acids include, but are not limited to, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, tryptophan, proline, and valine. Natural non-protein amino acids include, but are not limited to arginosuccinic acid, citrulline, cysteine sulfinic acid, 3,4-dihydroxyphenylalanine, homocysteine, homoserine, ornithine, 3-monoiodotyrosine, 3,5-diiodotyrosine, 3,5,5'-triiodothyronine, and 3,3',5,5'-tetraiodothyronine. Modified or unusual amino acids which can be used to practice the invention include, but are not limited to, D-amino acids, hydroxylysine,

4-hydroxyproline, an N-Cbz-protected amino acid, 2,4-diaminobutyric acid, homoarginine, norleucine, N-methylaminobutyric acid, naphthylalanine, phenylglycine, β -phenylproline, tert-leucine, 4-aminocyclohexylalanine, N-methyl-norleucine, 3,4-dehydroproline, N,N-dimethylaminoglycine, N-methylaminoglycine,
 5 4-aminopiperidine-4-carboxylic acid, 6-aminocaproic acid, trans-4-(aminomethyl)-cyclohexanecarboxylic acid, 2-, 3-, and 4-(aminomethyl)-benzoic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclopropanecarboxylic acid, and 2-benzyl-5-aminopentanoic acid.

In addition to abbreviations known by one skilled in the art for the designation
 10 of natural amino acids, abbreviations, known by one skilled in the art, used herein for modified and unusual amino acids are as follows: "Dpa" means diphenylalanine; "Cha" means cyclohexylalanine; "boroAlg-OH" means 2-amino-4-penten-boronic acid"; "Edans" means 5-[(2'-aminoethyl)amino]naphthylenesulfonic acid; "Abu ψ (COO)" mean 2-aminobutyric acid bonded through an ester bond; and
 15 "Dabcyl" means 4-[[4'-(dimethylamino)-phenyl]azo]benzoic acid.

The term "boronic acid" represents -B(OH)_2 . As used herein, the term "boronic acid ester" or "boron ester" is intended to represent esterified versions of boronic acid, for example, $\text{-BO}_2\text{R}$ and -B(OR)_2 , wherein $\text{-BO}_2\text{R}$ represents a boronic acid esterified by a diol moiety R and -B(OR)_2 represents a boronic acid esterified by
 20 two separate OR moieties. Examples of useful diols for esterification with the boronic acids are pinanediol, pinacol, 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, and 1,2-dicyclohexylethanediol.

The phrase "pharmaceutically acceptable" is employed herein to refer to those
 25 compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the
 30 disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic groups such as amines; and alkali or

organic salts of acidic groups such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived
5 from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic,
10 and isethionic.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the
15 appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

20 Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.) the compounds of the present invention may be delivered in prodrug form. Thus, the present invention is intended to cover prodrugs of the presently claimed compounds, methods of delivering the same and compositions containing the same. "Prodrugs" are intended
25 to include any covalently bonded carriers which release an active parent drug of the present invention *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound. Prodrugs include compounds
30 of the present invention wherein a hydroxy, amino, or sulfhydryl group is bonded to any group that, when the prodrug of the present invention is administered to a mammalian subject, it cleaves to form a free hydroxyl, free amino, or free sulfhydryl

group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups in the compounds of the present invention.

"Stable compound" and "stable structure" are meant to indicate a compound
5 that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

"Therapeutically effective amount" is intended to include an amount of a compound of the present invention or an amount of the combination of compounds claimed effective to reduce tumor growth rates, induce tumor regression or treat the
10 symptoms of cancer and immunological diseases in a host. The combination of compounds is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, *Adv. Enzyme Regul.* **1984**, *22*, 27-55, occurs when the effect of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In
15 general, a synergistic effect is most clearly demonstrated at suboptimal concentrations of the compounds.

As used herein, the term "treat" or "treating" refers to: (i) preventing a disease, disorder or condition from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it; (ii)
20 inhibiting the disease, disorder or condition, i.e., arresting its development; and (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

An "anti-cancer agent" as used herein includes known anti-cancer treatments such as radiation therapy or with cytostatic or cytotoxic agents, such as for example,
25 but not limited to, DNA interactive agents, such as cisplatin or doxorubicin; topoisomerase II inhibitors, such as etoposide; topoisomerase I inhibitors such as irinotecan or topotecan; tubulin interacting agents, such as paclitaxel, docetaxel or the epothilones; hormonal agents, such as tamoxifen; thymidilate synthase inhibitors, such as 5-fluorouracil; anti-metabolites, such as methotrexate; tyrosine kinase
30 inhibitors such as Iressa and Tarceva; angiogenesis inhibitors; EGF inhibitors; VEGF inhibitors; CDK inhibitors; Her1/2 inhibitors and monoclonal antibodies directed

against growth factor receptors such as erbitux (EGF), herceptin (Her2), or avastin (VEGF).

SYNTHESIS

5 The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. Preferred methods include, but are
10 not limited to, those described below. All references cited herein are incorporated by reference in their entirety.

 The compounds of this invention may be prepared using the reactions and techniques described in this section. The reactions are performed in solvents appropriate to the reagents and materials employed and are suitable for the
15 transformations being effected. Also, in the description of the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the experiment and workup procedures, are chosen to be the conditions standard for that reaction, which should be readily recognized by one skilled in the art. It is understood
20 by one skilled in the art of organic synthesis that the functionality present on various portions of the molecule must be compatible with the reagents and reactions proposed. Such restrictions to the substituents which are compatible with the reaction conditions will be readily apparent to one skilled in the art and alternate methods must then be used.

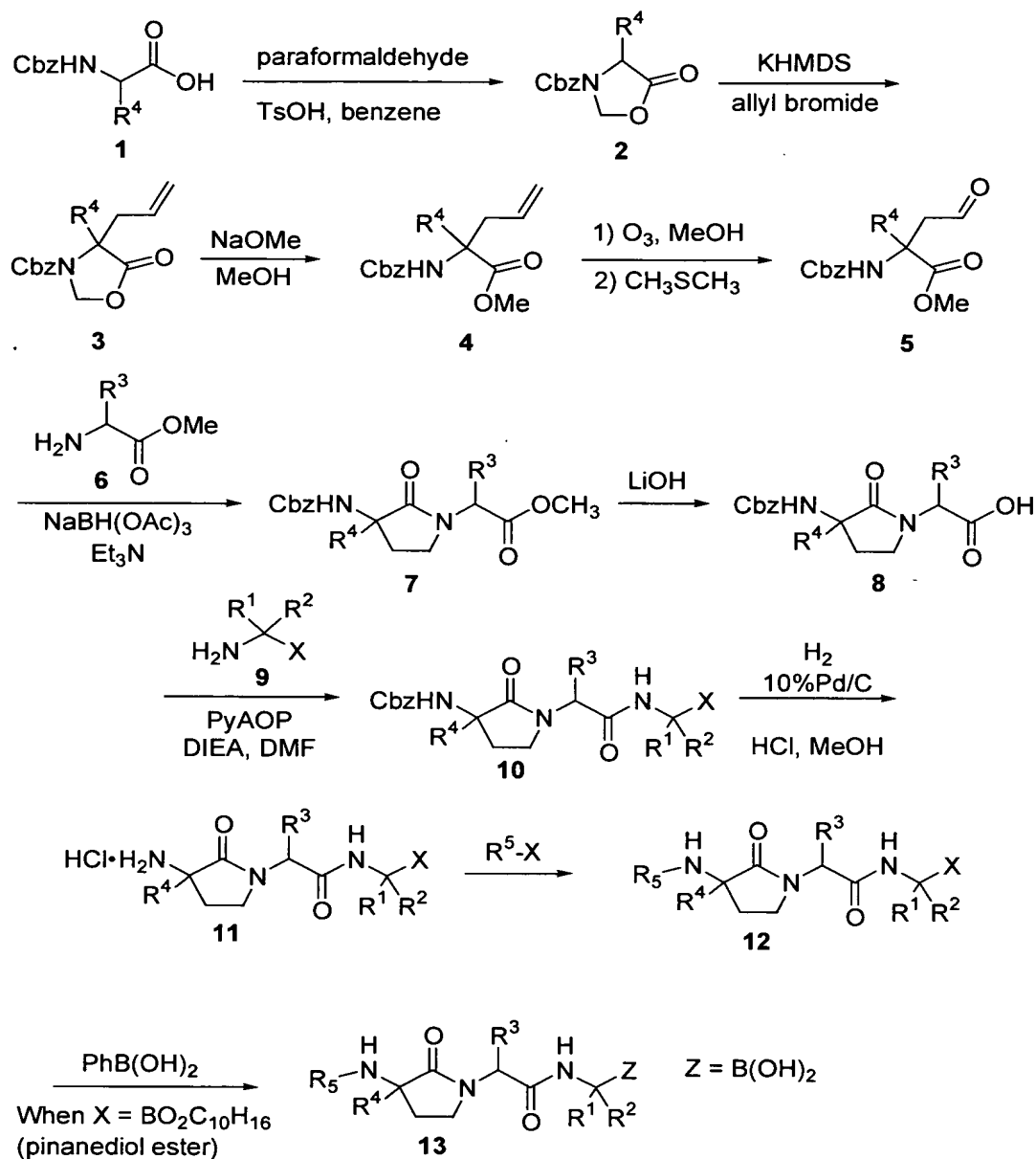
25 The compounds of this invention are intended to interact with the catalytic N-terminal threonine hydroxyl of the catalytic subunits of the 26S proteasome, and therefore incorporate an electrophilic moiety capable of such interaction. In the synthetic schemes below, this moiety, or its synthetic equivalent or precursor, is referred to as a "threonine trap" and is defined by formula 9.

30 A series of γ -lactams of formula 12 and 13 are prepared by the method outlined in Scheme 1. Cbz protected, R⁴-substituted amino acid 1 is treated with paraformaldehyde and *p*-toluenesulfonic acid to give oxazolidinone 2. Subsequent

alkylation with allyl bromide provides the racemic disubstituted oxazolidinone **3**. Treatment with sodium methoxide in methanol affords amino acid methyl ester **4**. The olefin in **4** is cleaved by ozonolysis to give aldehyde **5**. Reductive amination of aldehyde **5** with amino acid methyl ester **6**, followed by lactamization provides the
5 lactam **7**. Saponification of the methyl ester affords acid **8**, which is coupled to **9** (see subsequent discussion) using either the phosphonium salt PyAOP (Carpino, et al. *J. Chem. Soc., Chem. Commun.* **1994**, 201-203.) or by *in situ* formation of a mixed anhydride of acid **8** and subsequent aminolysis with **9**. Catalytic hydrogenation of the resulting **10** affords amine hydrochloride salt **11**, which may be acylated,
10 sulfonlated, reductively alkylated, etc. to provide **12** as a mixture of two diastereomers epimeric at the chiral center bearing substituent R₄. The diastereomeric mixture at this stage could be resolved using chiral HPLC to get two constituent diastereomers; **12R** and **12S**. Each of these esters can be converted into corresponding boronic acid **13**.

15

SCHEME 1



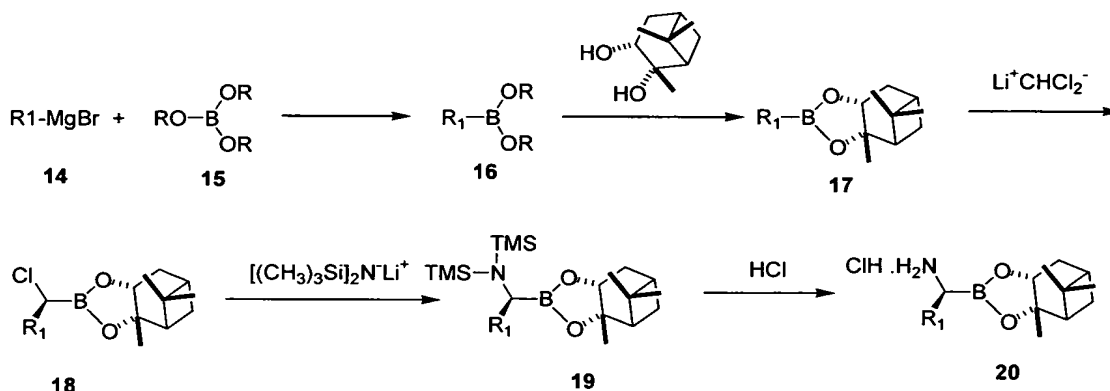
5 Many of the Cbz protected amino acids **1** and amino acid methyl esters **6** are commercially available or may be prepared from commercial amino acid derivatives by simple protecting group manipulations. Others may be synthesized in racemic form using the Strecker synthesis or amidomalonate synthesis. In addition, the Myers pseudoephedrine glycinamide alkylation method (Myers, A. G.; Gleason, J. L.; Yoon,
10 T; Kung, D. W.. *J. Am. Chem. Soc.* **1997**, *119*, 656-673) and the Evans electrophilic

azidation (Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011) may be used to prepare unnatural amino acids in enantiomerically pure form.

The threonine trap **9** may be either an α -amino boronic ester ($X = \text{BO}_2\text{R}$) or
 5 the reduced form of an α -keto amide ($X = \text{CH}(\text{OH})\text{CONHR}$) or other electrophilic carbonyl derivative known to one skilled in the art (Edwards, P. D.; Bernstein, P. R. *Medicinal Res. Reviews* **1994**, *14*, 127-194, and references cited therein). Scheme 2 shows the synthetic route to monosubstituted amino boronic esters **20** (For a general reference to synthesis of peptide boronic esters, see: Kettner, C.; Forsyth, T. *Houben-*
 10 *Weyl Methods of Organic Chemistry* **1999**, in press). Grignard reagent **14** is reacted with a trialkyl borate ester **15**, providing boronate **16**. Transesterification with (+)-pinanediol affords the cyclic ester **17**. This ester ultimately yields enantiomerically pure **20** with L-configuration. Substitution of pinacol for pinanediol yields racemic product. Homologation of **17** with the anion of dichloromethane gives the α -chloro
 15 boronic ester **18**. (Matteson, D. S.; Majumdar, D. *Organometallics* **1983**, *2*, 1529-1535) Displacement of chloride by lithium bis(trimethylsilyl)amide, gives silyl amine **19**, which is converted to the amine hydrochloride **20** with anhydrous HCl. (Matteson, D. S., Sadhu, K. M. *Organometallics* **1984**, *3*, 1284-1288.)

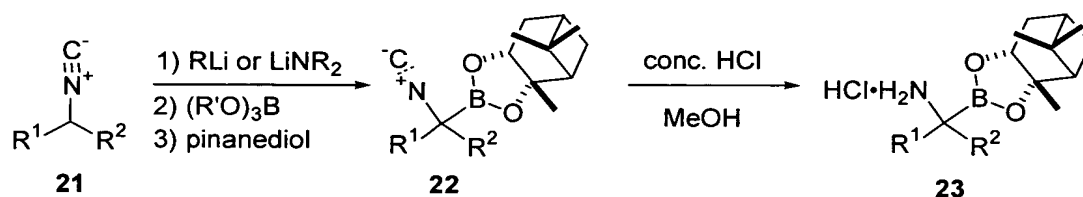
α,α -Disubstituted amino boronic esters **23** may be prepared as shown in
 20 Scheme 3. An isocyanide **21** (commercially available or synthesized by methods known to one skilled in the art. See for instance: Ugi, I.; et al. *Angew. Chem., Intl. Ed. Eng.* **1965**, *4*, 472.) is metallated with an alkyllithium or lithium dialkyl amide base (Hoppe, D. *Angew. Chem., Intl. Ed. Eng.* **1974**, *13*, 789-804 and reacted with a trialkyl borate ester. Transesterification with pinanediol affords α -isocyanoboronic
 25 ester **22**. Hydrolysis of **22** in conc. HCl/MeOH yields the α,α -disubstituted amino boronic ester **23**, which is a specific instance of formula **9** for which $X = \text{BO}_2\text{R}$ and neither R^1 nor R^2 are hydrogen.

SCHEME 2



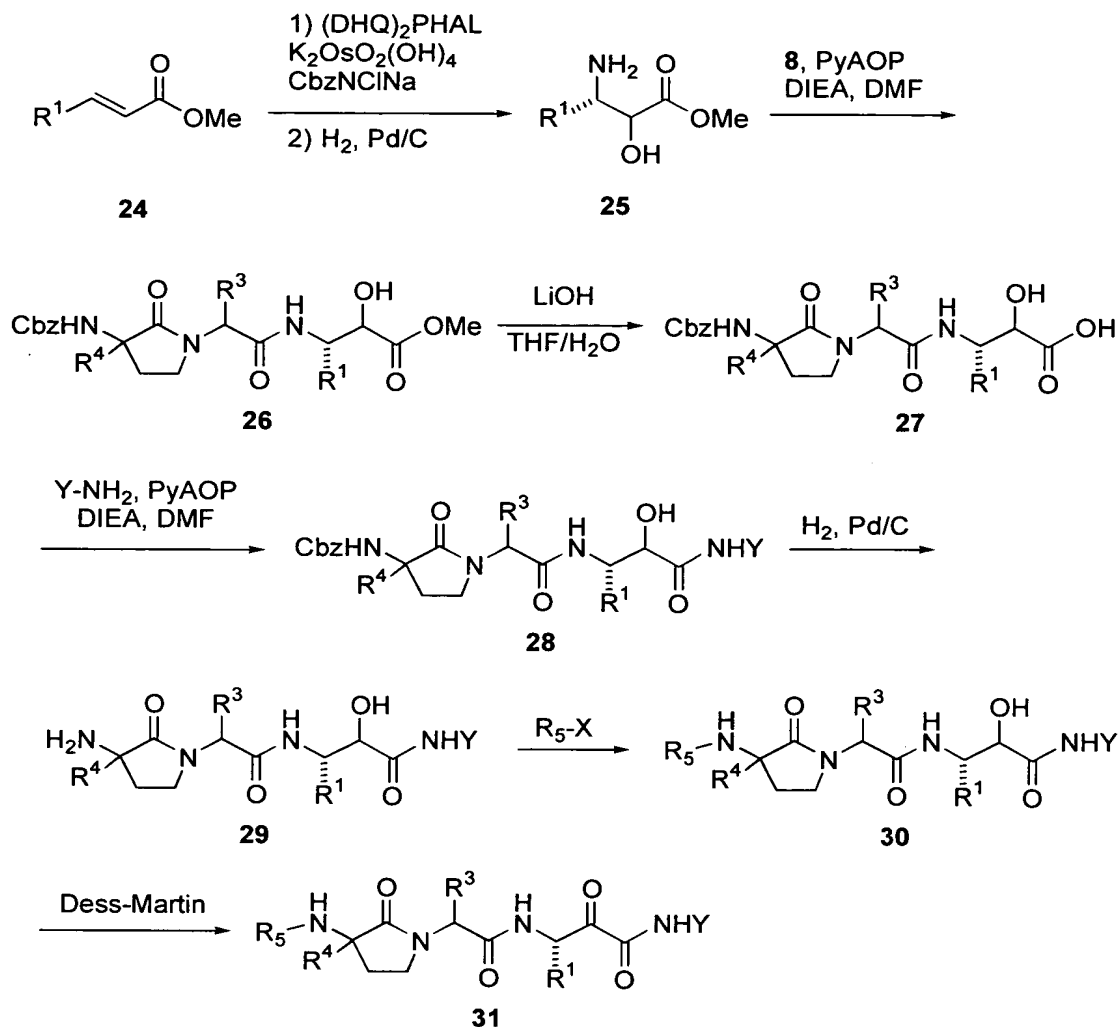
5

SCHEME 3



α -Ketoamides and other electrophilic ketone derivatives are generally introduced in the hydroxy form and oxidized to the active ketone form in the final synthetic step. Scheme 4 illustrates the synthesis of α -ketoamide γ -lactam peptidomimetics. Other electrophilic ketone derivatives may be prepared analogously (Edwards, P. D.; Bernstein, P. R. *Medicinal Res. Reviews* **1994**, *14*, 127-194, and references cited therein). R^1 substituted acrylate ester **24** is aminohydroxylated and subsequently deprotected to give amino alcohol **25** (Note that this structure is a specific instance of formula **9**, for which $X = \text{CH}(\text{OH})\text{COOMe}$). The amino alcohol is coupled to acid **8** to give **26**. Saponification with LiOH affords acid **27**, which is coupled to an amine Y-NH_2 , to give hydroxy amide **28**. Hydrogenation of the Cbz group, followed by acylation, sulfonylation, reductive amination, etc. of the resulting amine **29** provides **30**. Oxidation with Dess-Martin periodinane affords the α -keto amide **31** (a specific instance of formula **12**, for which $X = \text{COCONY}$).

SCHEME 4

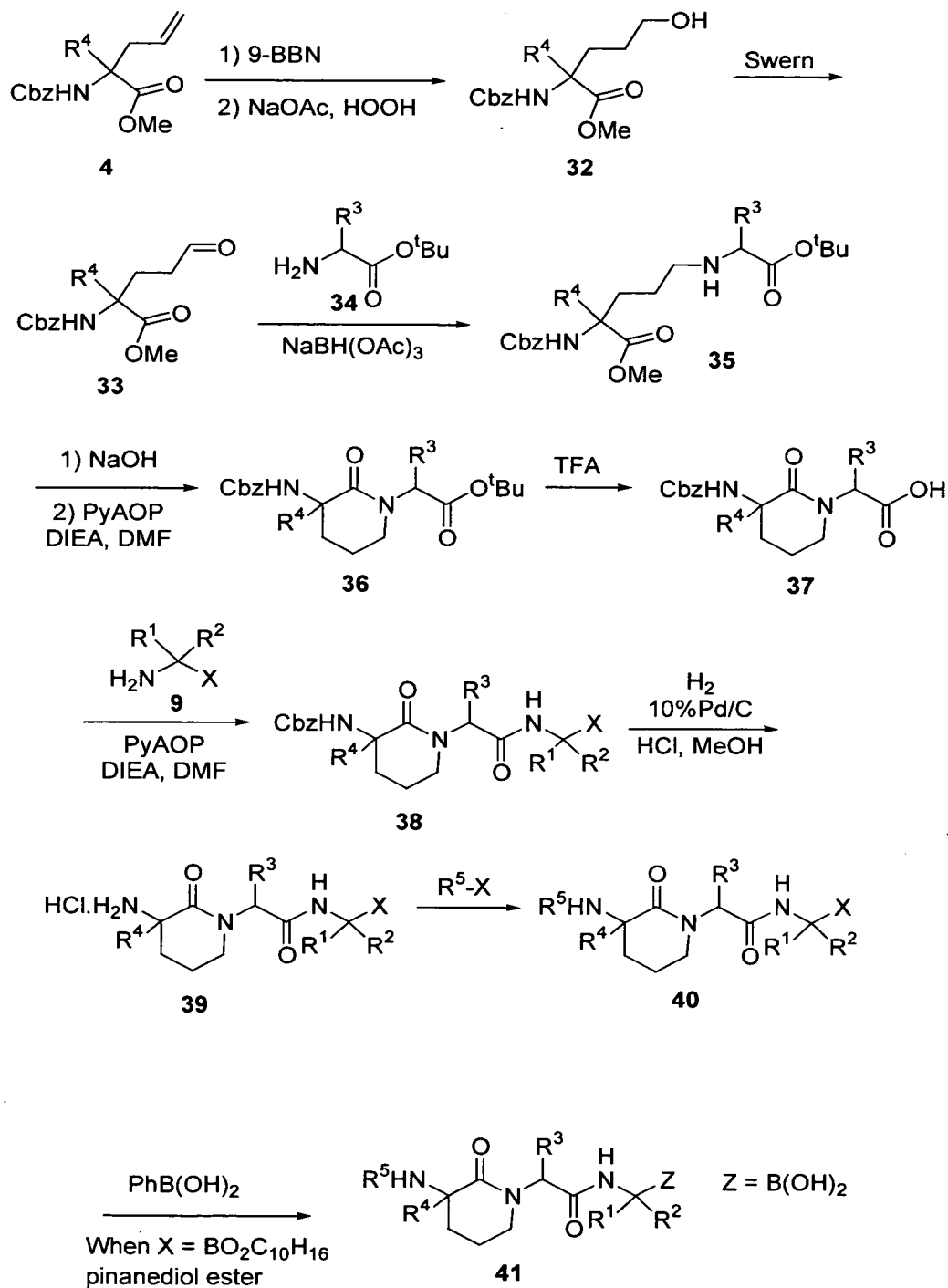


- 5 A series of δ -lactam derivatives of formula **40** and **41** are prepared by the method outlined in Scheme 5. Allylated, R⁴ substituted amino acid methyl ester **4**, prepared as shown in Scheme 1, is hydroborated and oxidized to alcohol **32**. Swern oxidation affords aldehyde **33**, which is reductively aminated with R³ substituted amino acid t-butyl ester **34** to afford amine **35**. Saponification of the methyl ester in
- 10 **35**, followed by cyclization affords lactam **36**. The t-butyl ester is removed with trifluoroacetic acid to give acid **37**. Coupling acid **37** to threonine trap **9** with PyAOP or via a mixed anhydride affords compounds of formula **38**. Catalytic hydrogenation provides amine hydrochloride **39**, which may be acylated, sulfonylated, reductively

alkylated, etc. to provide δ -lactams of formula **40** as a mixture of two diastereomers epimeric at the chiral center bearing substituent R_4 . The diastereomeric mixture at this stage could be resolved using chiral HPLC to get two constituent diastereomers; **40R** and **40S**. Each of these esters can be converted into corresponding boronic acid

- 5 41. Numerous amino acid t-butyl esters **34** are commercially available or may be synthesized by methods known to one skilled in the art (Roeske, R. *J. Org. Chem.* **1963**, 28, 1251-1253).

SCHEME 5

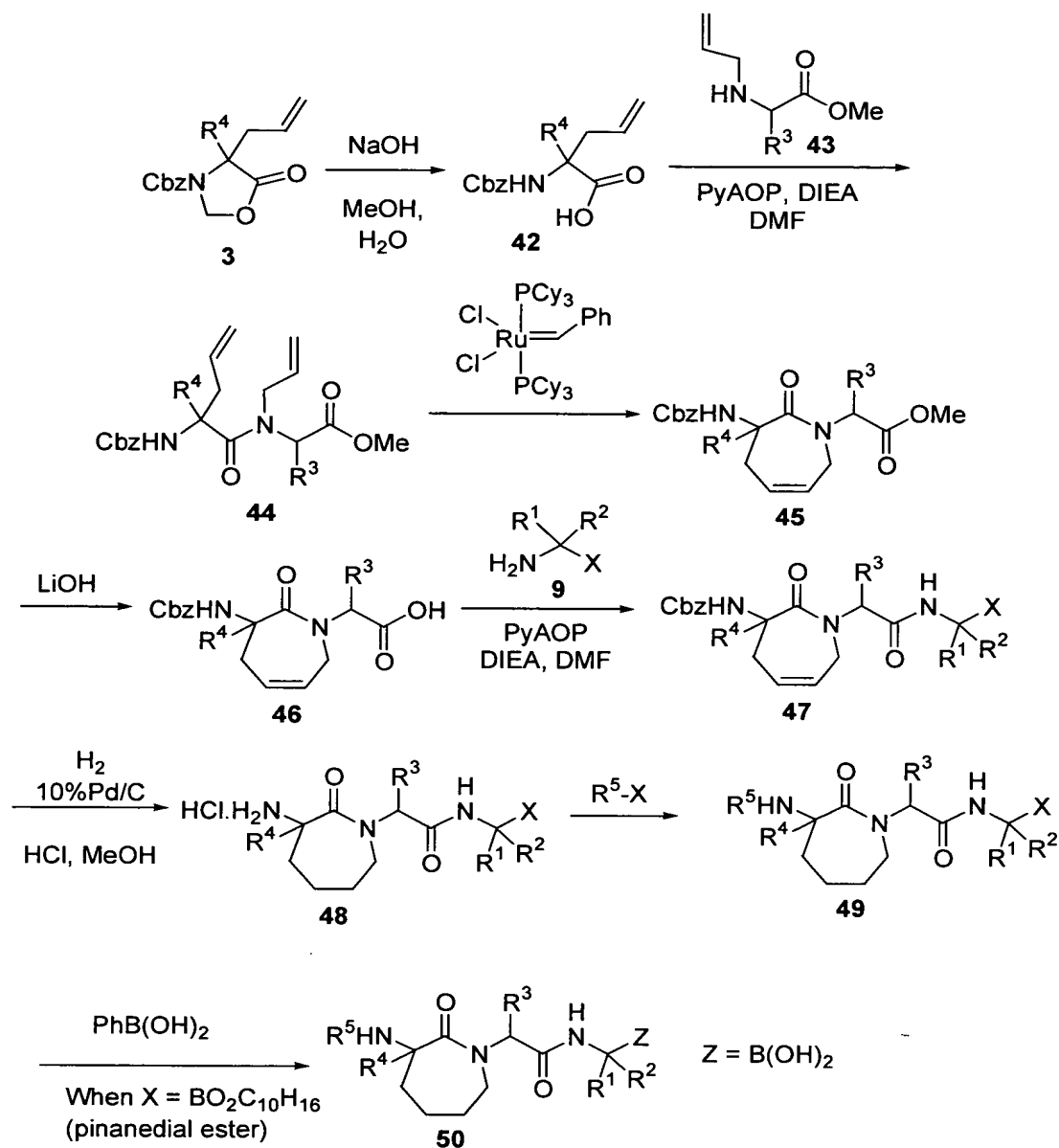


5

A series of ϵ -lactams of formula **49** and **50** may be synthesized by the method shown in Scheme 6. R^4 substituted oxazolidinone **3** is hydrolyzed to acid **42** with

NaOH. The acid is coupled to R³ substituted-N-allyl amino acid methyl ester **43** using activating reagents suitable for hindered peptide coupling reactions (Albericio, et al. *J. Org. Chem.* **1998**, *63*, 9678-9683. Wenschuh, H., et al. *Tetrahedron Lett.* **1996**, *37*, 5483-5486.) to afford dipeptide **44**. Ring closing olefin metathesis with ruthenium catalyst (Miller, S. J. et al. *J. Am. Chem. Soc.* **1996**, *118*, 9606.) affords the lactam **45**. The methyl ester in **45** is saponified to provide acid **46**. Coupling to serine trap **9** gives **47**. Catalytic hydrogenation removes the Cbz group and the olefin to provide amine hydrochloride **48**, which may be acylated, sulfonylated, reductively alkylated, etc. to provide δ -lactams of formula formula **49** as a mixture of two diastereomers epimeric at the chiral center bearing substituent R₄. The diastereomeric mixture at this stage could be resolved using chiral HPLC to get two constituent diastereomers; **40R** and **40S**. Each of these esters can be converted into corresponding boronic acid **50**. Alternatively, the olefin in **47** may be subjected to a variety of procedures (dihydroxylation, epoxidation followed by nucleophilic opening, etc.) to introduce substituents on the lactam ring prior to the final two steps of the synthesis. R³ substituted-N-allyl amino acid methyl esters **43** may be prepared from R³-substituted α -bromo esters ((Gribble, G. W.; Hirth, B. H. *J. Heterocyclic Chem.* **1996**, *33*, 719-726.)

SCHEME 6

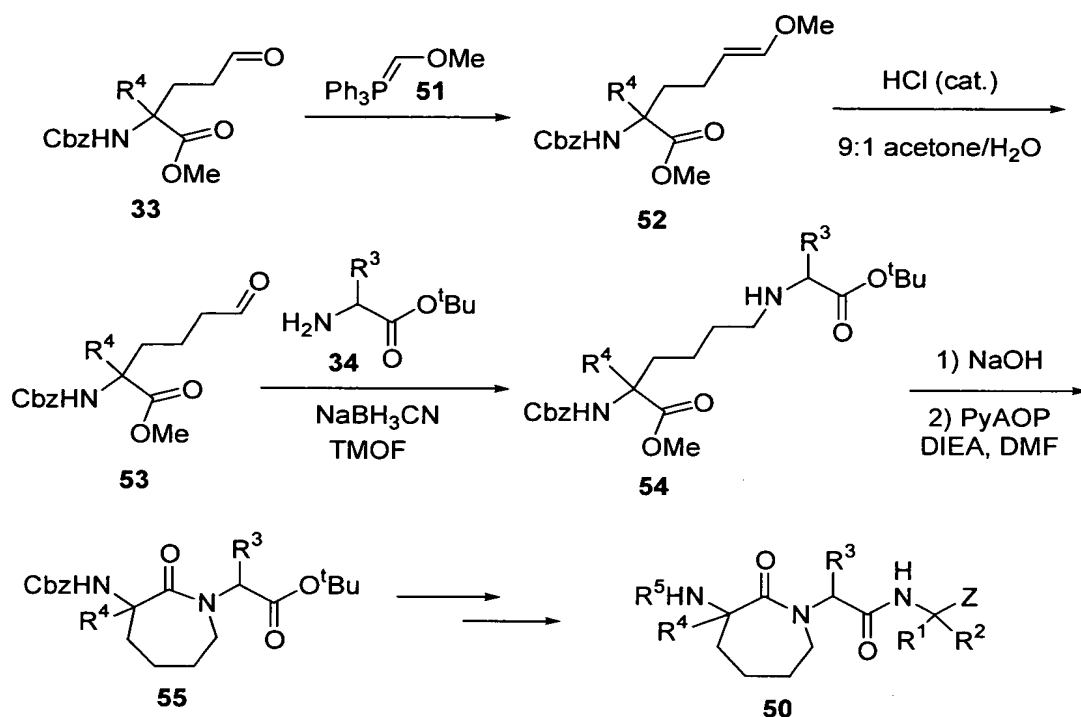


- 5 An alternative route to the series of ϵ -lactams of formula 49 is shown in Scheme 7. This route is applicable for cases in which R^3 and R^4 are too sterically demanding to allow coupling of 41 and 42 in Scheme 6. R^4 -substituted aldehyde 33 (see Scheme 5 for preparation) is treated with phosphonium ylide 51 to afford enol ether 52. The enol ether is hydrolyzed to aldehyde 53. The aldehyde is reductively aminated with amine 34 (see Scheme 5) using sodium cyanoborohydride in trimethyl
- 10

orthoformate to afford **54**. Saponification of the methyl ester, followed by cyclization affords lactam **55**. Lactam **54** may be transformed into **50** following the same procedure employed in Scheme 5.

5

SCHEME 7

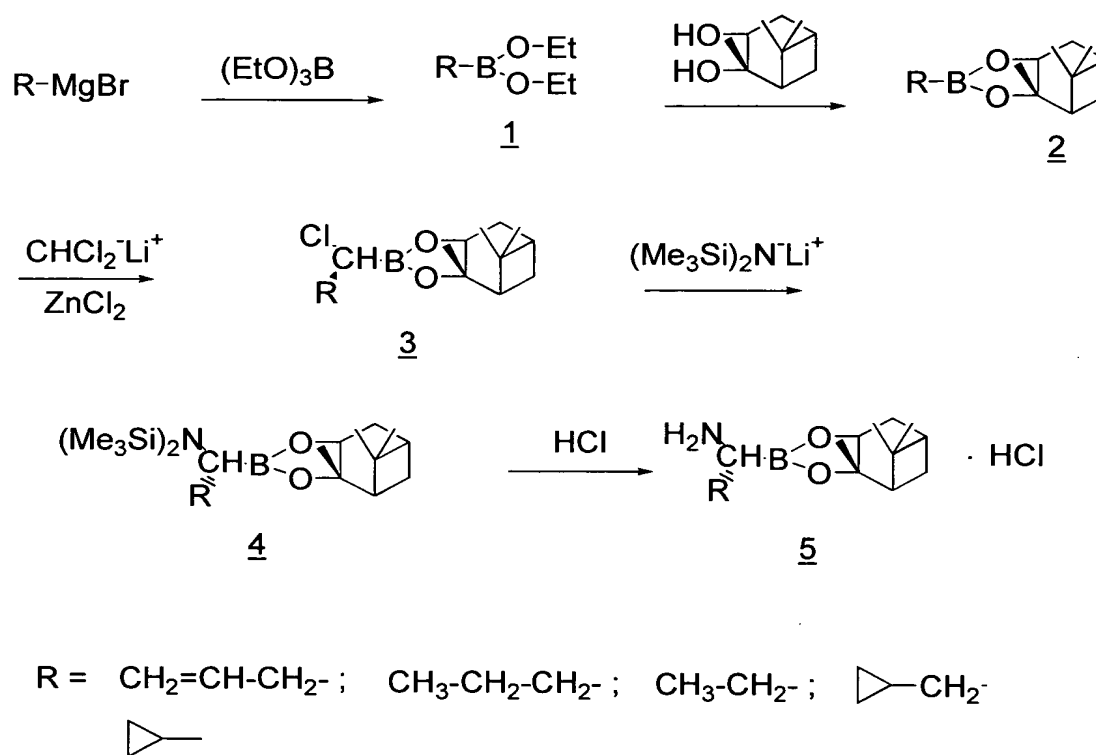
Preparation of α -aminoboronic acids intermediates

- 10 Preparation of α -aminoboronic acids are well known in the art. Scheme 8 shows the synthesis of α -aminoboronic acids containing sidechains where R is ethyl, allyl, vinyl, and cyclopropyl. A Grignard reagent is added to a trialkyl boronate to give a substituted dialkyl boronate. Transesterification with a suitable diol protecting group gives the boronate ester **2**. **2** is shown protected as the pinanediol ester.
- 15 Pinanediol is the preferred protecting group, but other diol protecting groups are known to those skilled in the art, for example, a C2 symmetrical diol such as (*R,R*)-2,3-butandiol and (*R,R*)-dicyclohexaneethanediol can also be used. The α -chloroalkyl intermediate **3** is obtained by the addition of the anion of methylene chloride to the boronic acid ester. $\text{Li}^+\text{CHCl}_2^-$ is prepared in situ by the addition of

LDA to a -78°C solution of the alkyl boronic acid ester in methylene chloride. Alternately, $\text{CHCl}_2\text{-Li}^+$ is prepared by reacting *n*-butyl lithium with methylene chloride at -100°C followed by the addition of the alkyl boronic acid 2. ZnCl_2 is added to more hindered alkyl boronic acid. 3 is treated with the lithium salt of hexamethyldisilazane to give the *bis*-silane protected amine 4. Compound 4 is treated with either anhydrous HCl or trifluoroacetic acid to give the amine 5 as a hydrochloride salt or trifluoroacetate salt.

SCHEME 8

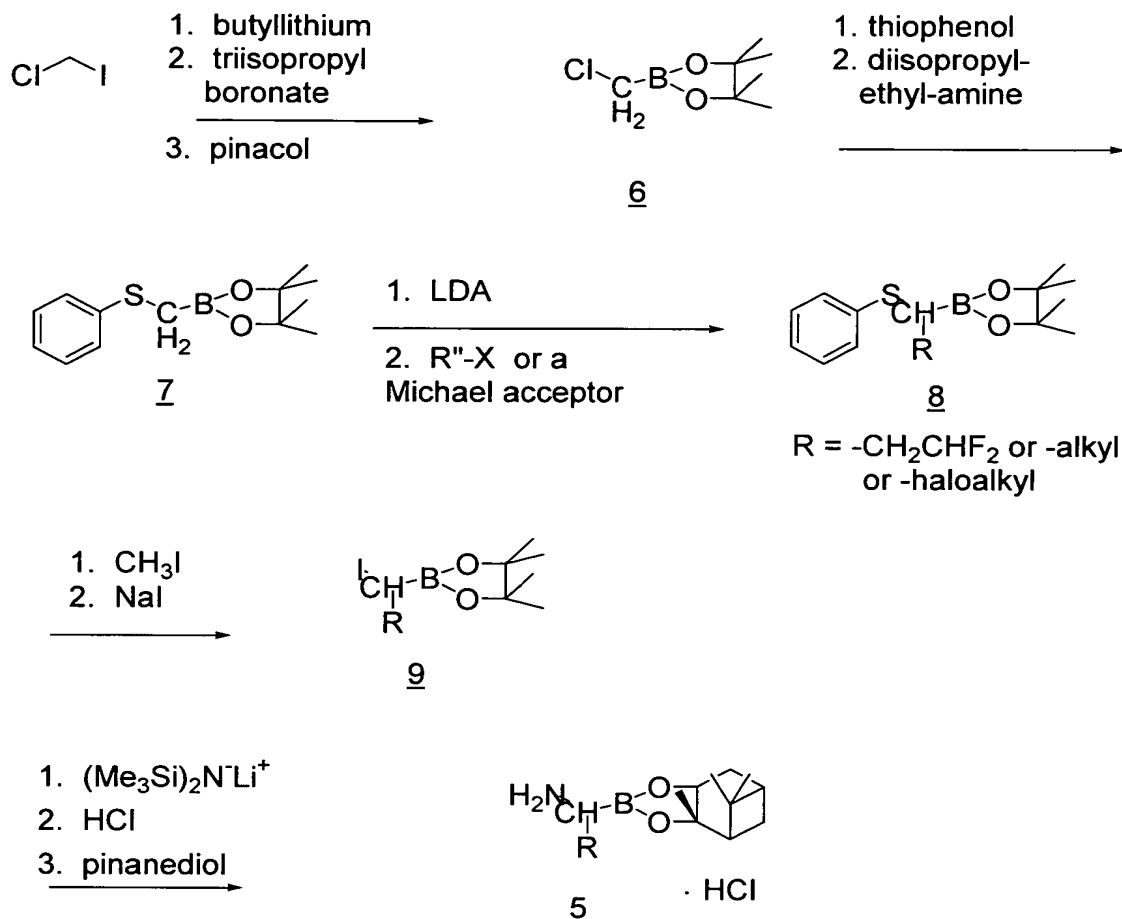
10



Scheme 8a outlines a method of preparing α -aminoboronic acids suitable for incorporation in to a peptide and applied as enzyme inhibitors. Matteson (Matteson and Majumdar *J. Organometallic Chem.* 170, 259-264, 1979; Matteson and Arne *Organometallics* 1, 280-288, 1982) discloses the preparation of α -haloboronic acids. Compound 6 is prepared by the method described by Sadhu and Matteson *Organometallics* 4, 1687-1689, 1985. Compound 6 is allowed to react with

thiophenol in presence of tertiary base to give the thiol ether 7. Alternately, 7 can be prepared by reacting the lithium salt of thioanisole with a trialkyl boronate as described by Matteson and Arne *Organometallics* 1, 280-288 (1982). 7 is treated with LDA followed by a hydrocarbon containing an electrophilic center. For this
5 reaction 1-bromo-2,2-difluoroethane was used to give a 2,2-difluoroethyl substituent 8. The α -aminoboronic acid 9 was obtained by treating 8 with methyl iodide or other suitable alkylating agent in the presence of iodide ion followed by lithium hexamethyldisilazane and HCl. In contrast to other procedures for preparing α -aminoboronic acids where the sidechain is introduced as a nucleophile or an alkene,
10 the sidechain substituent is an electrophile. This provides a method of preparing 2-amino-3,3-difluoropropyl boronic acid where conventional methods have failed.

SCHEME 8a



5 The chemistry outlined in Scheme 8a is readily applied by one skilled in the art to the synthesis of additional α -aminoboronic acids. After treatment of 7 with base to generate the anion at the α -position, a Michael acceptor can be added to synthesize additional more structurally diverse α -aminoboronic acids, for example, higher order alkyl halides which can be used to give more complex sidechains.

10 Scheme 8b illustrates the preparation of α -aminoboronic acids with hydroxy substituted side chains, boroSerine and boroThreonine. Both are synthesized as their benzyl protected form and incorporated into peptides. The benzyl protecting groups are removed by catalytic hydrogenation to give the final product. The synthesis of 2-benzyloxy-1-chloroethane boronic acids esters has been described (Matteson et al.

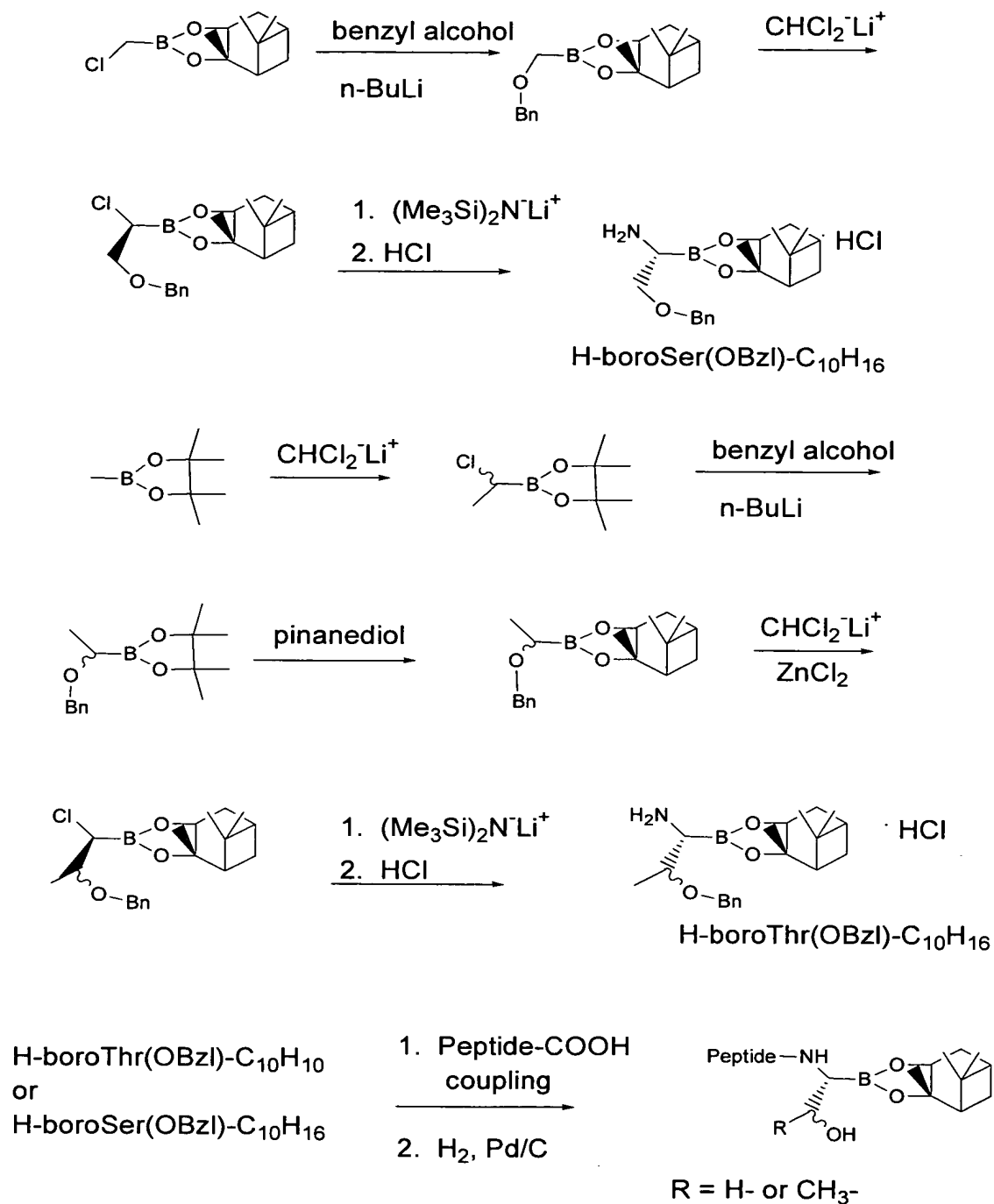
15 *Organometallics* 3, 1284-1288, 1984). For H-boroSer(OBzl)-C₁₀H₁₆, the α -

chloromethyl boronic acid is treated with the anion of benzyl alcohol to give the benzyl ether. Homologation with the anion of methylene chloride gives the α -chloro compound. It is readily converted to the α -aminoboronic acid by conventional procedures. Boro-threonine is prepared by a similar procedure except an α -chloroethyl boronic acid ester is prepared and converted to the benzyl protected alcohol.

5 Homologation with $\text{CHCl}_2\text{-Li}^+$ and treatment with $(\text{Me}_3\text{Si})_2\text{N-Li}^+$ and HCl gives H-boroThr(OBzl)- $\text{C}_{10}\text{H}_{16}$. The first series of reactions were conducted using the pinacol ester which resulted in the nonstereo specific introduction of the O-benzyl hydroxy group. This group can be introduced in the natural configuration *R*-

10 configuration by using (*S,S*)dicyclohexaneethanediol as a chiral directing boronic acid protecting group.

SCHEME 8b

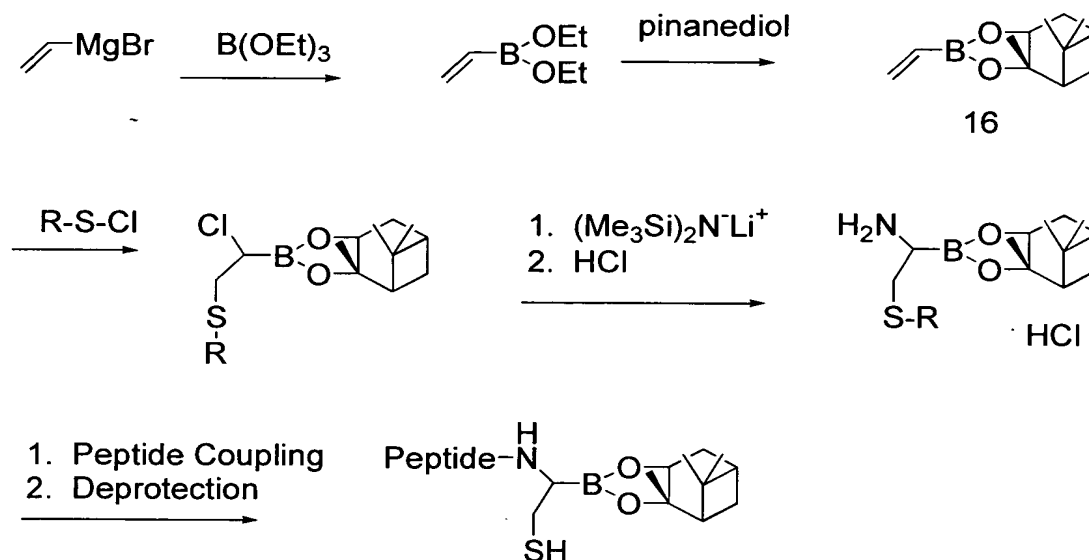


5 Scheme 8c describes the synthesis of boronic acid analogs of cysteine. Vinylmagnesium bromide is allowed to react with triethyl boronate to give vinylboronate diethyl ester. Transesterification with pinanediol gives the

corresponding ester **16**. Treatment of **16** with a sulfenyl chloride, for example phenyl sulfenyl chloride, gives the corresponding α -chloro-, α -thiol ether. The α -chloro group is readily converted to the amine using chemistry previously described (Scheme 8). Final deprotection of the thiol is achieved after incorporation of the amine in peptides. Additionally, the treatment of **16** with a thio sulfenyl chloride, for example phenyl thio sulfenyl chloride, followed by conversion to the amine using chemistry previously described (Scheme 8) gives the corresponding α -aminoboronic acid with a substituted disulfide side chain.

10

SCHEME 8c



It has been found, surprisingly, that compounds having the stereochemistry of Formula III display far superior activity compared with the other diastereomers.

Separation of the racemic material can be achieved by HPLC using a chiral column or by a resolution using a resolving agent such as camphonic chloride as in Steven D. Young, et al, *Antimicrobial Agents and Chemotherapy* **1995**, 2602-2605. A chiral compound of Formula (I) may also be directly synthesized using a chiral catalyst or a chiral ligand, e.g., Andrew S. Thompson, et al, *Tet. lett.* **1995**, 36, 8937-8940).

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

Abbreviations used in the examples are defined as follows: "1 x" for once, "2 x" for twice, "3 x" for thrice, "°C" for degrees Celsius, "rt" for room temperature, "eq" for equivalent or equivalents, "g" for gram or grams, "mg" for milligram or milligrams, "mL" for milliliter or milliliters, "M" for molar, "mmol" for millimole or millimoles, "min" for minute or minutes, "h" for hour or hours, "MS" for mass spectrometry, "NMR" for nuclear magnetic resonance spectroscopy, "¹H" for proton, "HPLC" for high pressure liquid chromatography, "tlc" for thin layer chromatography, "v/v" for volume to volume ratio, "atm" for atmosphere, "α", "β", "R", and "S" are stereochemical designations familiar to one skilled in the art.

EXAMPLE 1

(1*R*)-1-((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl)amino)-2-oxo-1-pyrrolidiny)propanoyl)amino)-3-butenylboronic acid (+)-pinanediol ester

(1a) A solution of Cbz-L-valine (4.88 g, 19.4 mmol), paraformaldehyde (0.84 g), and *p*-toluenesulfonic acid, (210 mg, 1.1 mmol) in benzene (160 mL) was refluxed for two h using a Dean-Stark apparatus. The solution was extracted with saturated sodium bicarbonate (2 x) and brine, dried (MgSO₄), and concentrated under reduced pressure to give the desired oxazolidinone as a colorless oil (4.82 g, 94%). NMR (¹H, CDCl₃) δ 7.37 (s, 5H), 5.60 (br s, 1H), 5.20 (m, 3H), 4.23 (br s, 1H), 2.37 (br s, 1H), 1.08 (d, 3H, J = 6.9), 1.01 (d, 3H, J = 6.6).

(1b) A 0.5 M solution of potassium bis(trimethylsilyl)amide in tetrahydrofuran (44 mL, 22 mmol) was added over 20 min to a solution of the material from (1a) (4.82 g, 18.3 mmol) in tetrahydrofuran (75 mL) at -78 °C. After 20 min, allyl bromide (3.2 mL, 37 mmol) was added dropwise, and the reaction was stirred at -78 °C for 2.5 h. The reaction was quenched with 10% potassium hydrogen sulfate (150 mL) and diluted with ethyl acetate (150 mL). The organic phase was extracted with 10% potassium hydrogen sulfate, saturated sodium bicarbonate, and brine, dried (Na₂SO₄), and concentrated under reduced pressure. The residue was

purified by chromatography on silica gel (ethyl acetate/hexane, 10:90) to give a colorless oil (3.8 g, 68%). MS found (M+H)⁺ = 304.

5 (1c) A 1 M solution of sodium methoxide in methanol (3 mL, 3 mmol) was added to the material from (1b) (0.61 g, 2.0 mmol) in methanol (5 mL). The reaction was refluxed for 1 h, quenched with acetic acid (0.165 mL, 2.9 mmol), and concentrated under reduced pressure. The residue was dissolved in dichloromethane, extracted with saturated sodium bicarbonate, dried (Na₂SO₄), and concentrated under reduced pressure to yield a colorless oil (0.63 g, 100%). MS found: (M+H)⁺ = 306.

10

(1d) Ozone was bubbled through a solution of the material from (1c) (0.593 g, 1.94 mmol) in methanol (20 mL) at -78 °C until a blue color persisted. Residual ozone was removed with a stream of oxygen. Dimethyl sulfide (0.6 mL, 8 mmol) was added, and the reaction mixture was allowed to warm to rt. After 2 h, the solution was concentrated under reduced pressure. The residue was dissolved in dichloromethane, extracted with water, dried (Na₂SO₄), and concentrated under reduced pressure to give a slightly yellow oil (0.65 g). The crude aldehyde was used without purification.

15

(1e) Sodium triacetoxyborohydride (0.649 g, 3.06 mmol) was added to a suspension of the material from (1d) (0.65 g, 1.9 mmol), L-cyclohexylalanine methyl ester hydrochloride (0.533 g, 2.4 mmol), and triethylamine (0.42 mL, 3.0 mmol) in 1,2-dichloroethane (10 mL) at 0 °C. The reaction was stirred overnight and allowed to warm to rt. The reaction was then refluxed for 5.5 h. The reaction mixture was diluted with dichloromethane and extracted with 1 M hydrochloric acid and saturated sodium bicarbonate. The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (ethyl acetate/hexane, 1:3) to provide a 1:1 mixture of lactam diastereomers as a waxy solid (0.67 g, 78%). MS found: (M+H)⁺ = 445, (M+Na)⁺ = 467.

25

30 (1f) A solution of the material from (1e) (0.33g, 0.74 mmol) in methanol (5 mL) was hydrogenated (1 atm, balloon) over 10% palladium on carbon (85 mg) for 2 h. The solution was filtered through Celite and concentrated under reduced pressure to

provide the desired product (0.225 g, 98%). MS found: $(M+H)^+ = 311$, $(M+Na)^+ = 333$.

(1g) Hunig's base (0.17 mL, 1.0 mmol) was added to a solution of the material from (1f) (0.126 g, 0.407 mmol), *N*-(pyrazine-2-carbonyl)-L-valine (0.109 g, 0.488 mmol), and PyAOP (0.261 g, 0.50 mmol) (Carpino, et al. *J. Chem. Soc., Chem. Commun.* **1994**, 201-203.) in dichloromethane at rt. After stirring overnight, the reaction was quenched with half saturated sodium carbonate (5 mL) and extracted with ethyl acetate. The organic phase was concentrated onto silica gel (500 mg) and purified by chromatography over silica gel (ethyl acetate/hexanes, 1:1) to give the desired product as a single diastereomer (74 mg, 35%). MS found: $(M+H)^+ = 516$, $(M+Na)^+ = 538$.

(1h) Lithium hydroxide monohydrate (10 mg, 0.24 mmol) was added to a solution of the material from (1g) (74 mg, 0.14 mmol) in a mixture of dimethoxyethane (1.5 mL) and water (1.0 mL) at 0 °C. The reaction was stirred for 30 min and quenched with 1 M hydrochloric acid (0.5 mL). The solution was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to provide the desired product as a colorless oil (66 mg, 92%). MS found: $(M-H)^- = 500$.

(1i) Isobutyl chloroformate (0.012 mL, 0.092 mmol) was added to a solution of the material from (1h) (41 mg, 0.082 mmol) and *N*-methyl morpholine (0.012 mL, 0.11 mmol) in tetrahydrofuran (1 mL) at -20 °C. After 10 min, a solution of the (+)-pinanediol ester of L-boroallylglycine hydrochloride salt (35 mg, 0.12 mmol) in dichloromethane (1.5 mL) was added dropwise, followed by Hunig's base (0.042 mL, 0.24 mmol). The reaction was stirred for 1.5 h and allowed to warm to rt. The reaction mixture was concentrated under reduced pressure and the residue purified by chromatography on silica gel (ethyl acetate/hexane gradient, 1:4 to 4:1) to give the desired boronic ester as an amorphous solid (41 mg, 69%). MS found: $(M+Na)^+ = 755.5$.

EXAMPLE 2

(1*R*)-1-((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl}amino)-2-oxo-1-piperidinyl)propanoyl}amino)-3-butenylboronic acid (+)-pinanediol ester

(2a) A 0.5 M solution of 9-borabicyclo(3.3.1)nonane in tetrahydrofuran (9.9 mL, 5 mmol) was added to a solution of the material from (1b) (1.01 g, 3.34 mmol) in tetrahydrofuran (15 mL) at 0 °C. The reaction was allowed to warm to rt and stir for 5 h before being quenched at 0 °C with a solution of sodium acetate (3.4 g) and 30% hydrogen peroxide (4 mL) in water (20 mL). The reaction was diluted with ethyl acetate and extracted with brine. The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (ethyl acetate/hexane 3:10) to provide the desired alcohol as a colorless oil (0.86 g, 80%). MS found: (M+NH₄)⁺ = 339.

(2b) Dimethyl sulfoxide (0.60 mL, 7.8 mmol) was added dropwise to a solution of oxalyl chloride (0.342 mL, 3.9 mmol) in dichloromethane (15 mL) at -78 °C. After 10 min, a solution of the material from (2a) (0.840 g, 2.62 mmol) in dichloromethane (5 mL) was added dropwise. After an additional 15 min, Hunig's base (2.2 mL, 13 mmol) was added dropwise. The reaction mixture was stirred 30 min at -78 °C and 3 h at 0 °C. The reaction was quenched with water. The organic phase was washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure to afford the crude aldehyde as a yellow oil (0.911 g). The material was used without further purification.

(2c) Following a procedure analogous to that used in the preparation of (1e), the aldehyde from (2b) (0.911 g, 2.6 mmol) was reacted with L-cyclohexylalanine methyl ester hydrochloride. Silica gel chromatography (ethyl acetate/hexane, 1:4) provided the desired product (1.05 g, 82%) as a 1:1 mixture of diastereomers. MS found: (M+H)⁺ = 489.

(2d) A 1.2 M solution of sodium methoxide in methanol (0.60 mL, 0.72 mmol) was added to a solution of the material from (2c) (318 mg, 0.651 mmol) in methanol (6 mL). The reaction was stirred at rt for 8 h, quenched with acetic acid (0.045 mL, 0.79 mmol), and concentrated under reduced pressure. The residue was purified by silica gel chromatography (methanol/dichloromethane gradient, 1:20 to 1:5) to yield the desired lactam (111 mg, 37%) as a colorless solid.

(2e) Following a procedure analogous to that used in step (1f), the material from step (2d) (0.127 g, 0.278 mmol) was hydrogenated to yield the crude product as a colorless oil (79.5 mg, 88%), which was used without further purification.

(2f) Following a procedure analogous to (1g), the material from (2e) (48 mg, 0.15 mmol) was coupled to *N*-(pyrazine-2-carbonyl)-L-valine with PyAOP and Hunig's base, providing the desired product as a single diastereomer (29 mg, 36%). MS found: (M+Na)⁺ = 552.

(2g) Following a procedure analogous to (1h), the methyl ester from (2f) (29 mg, 0.054 mmol) was saponified with lithium hydroxide to provide the desired acid (29 mg, 100%). MS found: (M-H)⁻ = 514.

(2h) Following a procedure analogous to (1i), the acid from step (2g) (28 mg, 0.054 mmol) was coupled to L-boroallylglycine hydrochloride salt using isobutylchloroformate. Silica gel chromatography (ethyl acetate/hexanes gradient, 1:4 to 4:1) of the crude material provided the desired boronic ester (5 mg, 12%) as an amorphous solid. MS found: (M+Na)⁺ = 769.5.

EXAMPLE 3

(1R)-1-(((3-(((methylsulfonyl)amino)-2-oxohexahydro-1H-azepin-1-yl)acetyl)amino)propylboronic acid (+)-pinanediol ester

(3a) Hunig's base (1.4 mL, 8.2 mmol) was added to a solution of *N*-Boc-D/L-allylglycine (0.645 g, 3.00 mmol), *N*-allylglycine ethyl ester (0.720 g, 5.0 mmol)

(Gribble, G. W.; Hirth, B. H. *J. Heterocyclic Chem.* **1996**, *33*, 719-726.), and PyAOP (2.04 g, 3.91 mmol) in dimethylformamide (10 mL). After stirring 3 h at rt, the reaction mixture was quenched by addition of methanol and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, extracted with saturated sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate/hexane, 1:3) to afford the desired dipeptide as a crystalline solid (0.887 g, 87%) MS found: $(\text{M}+\text{Na})^+ = 363$.

10 (3b) Bis(tricyclohexylphosphine)dichlororuthenium benzylidene catalyst (21 mg, 0.025 mmol) was added to a refluxing solution of the dipeptide from (3a) (174 mg, 0.512 mmol) in dichloromethane (50 mL) under argon. After 3 h, the reaction mixture was cooled to rt and concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate/hexane, 1:4) to give
15 the desired lactam (133 mg, 83%) as a crystalline solid. MS found: $(\text{M}+\text{Na})^+ = 335$.

 (3c) The material from (3b) (133 mg, 0.426 mmol) was hydrogenated using a procedure analogous to that of (1f), except that ethanol was employed as the solvent, giving the desired product (0.147g), which was used without further purification. MS
20 found: $(\text{M}+\text{Na})^+ = 337$.

 (3d) The material from (3c) (134 mg, 0.43 mmol) was saponified using a procedure analogous to that of (1h), except that tetrahydrofuran replaced dimethoxyethane as solvent, giving the desired acid as a colorless solid (0.121 g,
25 99%) MS found: $(\text{M}-\text{H})^- = 285$.

 (3e) Hunig's base (0.212 mL, 1.24 mmol) was added dropwise to a solution of the acid from (3d) (117 mg, 0.410 mmol), L-boro-2-aminobutyric acid hydrochloride (114 mg, 0.417 mmol), and PyAOP (216 mg, 0.414 mmol) in
30 dimethylformamide (2 mL). After 40 min, the reaction was diluted with ethyl acetate and extracted with 5% sodium bicarbonate and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica

gel chromatography (ethyl acetate/hexane, 4:1) to afford the desired boronic ester as an amorphous solid (0.155 g, 75%). MS found: $(M+Na)^+ = 528$.

5 (3f) A 4M solution of hydrochloric acid in dioxane (4 mL, 16 mmol) was added to the material from (3e) (141 mg, 0.28 mmol). The reaction was stirred for 2 h at rt and concentrated under reduced pressure to give the desired product as a colorless solid (133 mg), which was used without further purification. MS found: $(M+H)^+ = 406$.

10 (3g) Triethylamine (0.028 mL, 0.201 mmol) was added dropwise to a suspension of the material from (3f) (31 mg, 0.07 mmol) and methanesulfonyl chloride (0.010 mL, 0.135 mmol). After stirring for 18 h, the reaction was diluted with dichloromethane and extracted with 5% sodium bicarbonate. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was
15 purified by HPLC on a C18 reverse phase (acetonitrile/water gradient, 4:6 to 8:2) to afford the desired sulphonamide as an amorphous solid (18.5 mg, 56%). MS found: $(M+H)^+ = 484$.

EXAMPLE 4

20 **(1R)-1-(((2S)-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidiny)-3-cyclohexylpropanoyl)amino}propylboronic acid (+)-pinanediol ester hydrochloride**

(4a) The 1:1 mixture of lactam diastereomers (6.5 g, 14 mmol) from (1e)
25 was recrystallized from ethyl acetate/hexanes to give a single lactam diastereomer (1.9 g, 30%). $(\alpha)_D^{25} = -16.8^\circ$ ($C = 0.280$, methanol).

(4b) The lactam ester from (4a) (1.11 g, 2.5 mmol) was saponified using a procedure analogous to that of (1h), except that tetrahydrofuran replaced
30 dimethoxyethane as solvent. The crude acid was obtained as a colorless foam (1.06 g, 100%) and was used in the next step without purification. MS found $(M+H)^+ = 431$.

(4c) The material from (4b) (1.06 g, 2.5 mmol) was coupled to L-boro-2-aminobutyric acid hydrochloride salt using a procedure analogous to (1i). The crude material was purified by silica gel chromatography (ethyl acetate/hexane, 1:1) to give the desired boronic ester (0.85 g, 52%). MS found $(M+H)^+ = 650$.

5

(4d) A solution of the boronic ester from (4c) (400 mg, 0.616 mmol) in a mixture of methanol (10 mL), 4M hydrochloric acid in dioxane (5 mL) and dioxane (30 mL) was hydrogenated (48 psi) over 10% palladium on carbon for 3 h at rt. The reaction mixture was filtered and concentrated under reduced pressure to afford the
10 desired amine hydrochloride (340 mg, 100%). MS found: $(M+H)^+ = 516$.

EXAMPLE 5

**(1*R*)-1-(((2*S*)-2-{3-(((1,1'-biphenyl)-4-ylsulfonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)-amino)-propylboronic acid (+)-pinanediol
15 ester**

(5a) 4-Biphenylsulfonyl chloride (5 mg, 0.02 mmol) was added to a solution of the amine hydrochloride from (4d) (11 mg, 0.020 mmol), 4-dimethylamino pyridine (0.6 mg, 0.005 mmol), and triethylamine (0.012 mL, 0.086 mmol) in a
20 mixture of 1,2-dichloroethane (0.2 mL) and ethyl acetate (0.1 mL). The reaction mixture was heated at 57 °C overnight and quenched by addition of water. The mixture was extracted with dichloromethane and the organic phase concentrated under reduced pressure. The residue was dissolved in acetonitrile, filtered, and purified by HPLC (acetonitrile/water gradient) to afford the desired sulfonamide (2
25 mg, 14%). MS found: $(M+H)^+ = 732$.

EXAMPLE 6

(1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-2-oxo-3-((4-propylphenyl)sulfonyl)amino)-1-pyrrolidinyl)propanoyl)-amino}propylboronic acid (+)-pinanediol ester

5

(6a) Using a procedure analogous to (5a) the amine hydrochloride from (4d) (11 mg, 0.020 mmol) was coupled to 4-propylphenylsulfonyl chloride to provide the desired sulfonamide (7 mg, 50%). MS found: $(M+H)^+ = 698.5$.

10

EXAMPLE 7

(1*R*)-1-(((2*S*)-3-cyclohexyl-2-{3-isopropyl-3-((1-naphthylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}-propanoyl)amino)propylboronic acid (+)-pinanediol ester

(7a) Using a procedure analogous to (5a) the amine hydrochloride from (4d) (11 mg, 0.020 mmol) was coupled to 1-naphthylsulfonyl chloride to provide the desired sulfonamide (3.3 mg, 23%). MS found: $(M+H)^+ = 706.5$.

15

EXAMPLE 8

(1*R*)-1-(((2*S*)-2-{3-((anilincarbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl}-3-cyclohexylpropanoyl)-amino)propylboronic acid (+)-pinanediol ester

20

(8a) Using a procedure analogous to (5a) the amine hydrochloride from (4d) (11 mg, 0.020 mmol) was coupled to phenylisocyanate to provide the desired sulfonamide (10 mg, 79%). MS found: $(M+H)^+ = 635.5$.

25

EXAMPLE 9

(1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((3-methylphenyl)sulfonyl)amino)-2-oxo-1-pyrrolidinyl)propanoyl)amino}propylboronic acid (+)-pinanediol ester

30

(9a) Using a procedure analogous to (5a) the amine hydrochloride from (4d) (11 mg, 0.020 mmol) was coupled to 3-methylphenylsulfonyl chloride to provide the desired sulfonamide (5.1 mg, 38%). MS found: $(M+H)^+ = 670.5$.

EXAMPLE 10

(1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-isopropyl-3-(((3-methylphenyl)sulfonyl)amino)-2-oxo-1-pyrrolidinyl)propanoyl)amino}propylboronic acid

5

(10a) Phenylboronic acid (40 mg, 0.32 mmol) was added to the boronic ester from (9a) (16 mg, 0.024 mmol) in a well-stirred mixture of dichloromethane (0.2 mL) and water (0.2 mL). The reaction mixture was stirred overnight. The organic layer was concentrated under reduced pressure and the residue purified by preparative tlc (chloroform/methanol, 9:1) to afford the desired boronic acid as a colorless solid (4 mg, 31%). MS found: (M-H)⁻ = 534.

10

EXAMPLE 11

(1*R*)-1-(((3-(((benzyloxy)carbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

15

(11a) Using a procedure analogous to (1e), the aldehyde from (1d) (0.45 g, 1.46 mmol) was reductively aminated with L-phenylglycine hydrochloride in the presence of sodium triacetoxy borohydride and triethylamine. Silica gel chromatography (ethyl acetate/hexane gradient, 1:9 to 3:2) afforded the desired product as a 1:1 mixture of diastereomers. MS found: (M+H)⁺ = 425.

20

(11b) Using a procedure analogous to (1h), except that tetrahydrofuran replaced dimethoxyethane as solvent, the material from (11a) was saponified with lithium hydroxide. The acid (140 mg, 23% over two steps) was obtained and used in the subsequent step without purification.

25

(11c) Using a procedure analogous to (3e), the acid from (11b) (0.135 g, 0.33 mmol) was coupled to L-boro-2-aminobutyric acid hydrochloride in the presence of PyAOP and Hunig's base. Silica gel chromatography (ethyl acetate/hexane, 3:10), afforded the desired boronic ester as a 1:1 mixture of diastereomers (0.180 g, 90%). MS found: (M+H)⁺ = 630

30

EXAMPLE 12

(1*R*)-1-(((3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester hydrochloride

(12a) The material from (11c) (0.185 g, 0.29 mmol) was hydrogenated (1 atm, balloon) in a mixture of concentrated hydrochloric acid (0.075 mL, 0.88 mmol) and methanol (10 mL) for 1 h at rt. The solution was filtered through Celite and concentrated under reduced pressure to give the desired amine hydrochloride (136 mg, 95%). MS found $(2M+H)^+ = 991$.

EXAMPLE 13

(1*R*)-1-(((3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

(13a) Using a procedure analogous to (5a), the amine hydrochloride from (12a) (20 mg, 0.038 mmol) was coupled to methane sulfonyl chloride. The crude product was purified by HPLC (acetonitrile:water gradient) to afford the desired product. MS found $(M+H)^+ = 574$.

EXAMPLE 14

(1*R*)-1-(((3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)-amino)-1-pyrrolidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

(14a) Using a procedure analogous to (5a), the amine hydrochloride from (12a) (30 mg, 0.056 mmol) was coupled to 4-propylphenylsulfonyl chloride. The crude product was purified by HPLC (acetonitrile:water gradient) to afford the desired product. MS found $(M+H)^+ = 678$.

EXAMPLE 15

(1*R*)-1-(((2*S*)-2-(3-(((benzyloxy)carbonyl)amino)-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl)-amino}propylboronic acid (+)-pinanediol ester

5 (15a) Using a procedure analogous to (1e), the aldehyde from (1d) (0.57 g, 1.85 mmol) was reductively aminated with L-leucine hydrochloride in the presence of sodium triacetoxy borohydride and triethylamine. Silica gel chromatography afforded the desired product (0.45 g, 61%) as a 1:1 mixture of diastereomers. MS found: (M+H)⁺ = 512.

10

 (15b) Using a procedure analogous to (1h), except that tetrahydrofuran replaced dimethoxyethane as solvent, the material from (15a) (0.45 g, 1.11 mmol) was saponified with lithium hydroxide. The acid (433 mg, quantitative) was obtained and used in the subsequent step without purification.

15

 (15c) Using a procedure analogous to (3e), the acid from (15b) (0.080 g, 0.205 mmol) was coupled to L-boro-2-aminobutyric acid hydrochloride in the presence of PyAOP and Hunig's base. Silica gel chromatography (ethyl acetate/hexane, 1:1), afforded the desired boronic ester (120 mg, 95%) as a 1:1 mixture of diastereomers. MS found: (M+H)⁺ = 610

20

EXAMPLE 16

(1*R*)-1-(((2*S*)-2-(3-amino-3-isopropyl-2-oxo-1-pyrrolidinyl)-4-methylpentanoyl)amino}propylboronic acid (+)-pinanediol ester hydrochloride

25

 (16a) The material from (15c) (0.120 g, 0.2 mmol) was hydrogenated using a procedure analogous to (12a) to provide the desired amine hydrochloride (90 mg, 95%). MS found (M+H)⁺ = 476.

EXAMPLE 17

(1*R*)-1-(((2*S*)-2-{3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-pyrrolidinyl}-4-methylpentanoyl)amino)propylboronic acid (+)-pinanediol ester

5 (17a) Using a procedure analogous to (5a), the amine hydrochloride from (16a) (21 mg, 0.041 mmol) was coupled to methane sulfonyl chloride. The crude product was purified by HPLC (acetonitrile:water gradient) to afford the desired product. MS found (M+H)⁺ = 554.

EXAMPLE 18

10 **(1*R*)-1-(((2*S*)-2-(3-isopropyl-2-oxo-3-(((4-propylphenyl)sulfonyl)amino)-1-pyrrolidinyl)-4-methylpentanoyl)amino)propylboronic acid (+)-pinanediol ester**

15 (18a) Using a procedure analogous to (5a), the amine hydrochloride from (16a) (20 mg, 0.039 mmol) was coupled to 4-propylphenylsulfonyl chloride. The crude product was purified by HPLC (acetonitrile:water gradient) to afford the desired product. MS found (M+H)⁺ = 658.

EXAMPLE 19

20 **(1*R*)-1-(((2*S*)-3-cyclohexyl-2-(3-ethyl-3-(((2*S*)-3-methyl-2-((2-pyrazinylcarbonyl)amino)butanoyl)amino)-2-oxo-1-pyrrolidinyl)propanoyl)amino)-3-butenylboronic acid (+)-pinanediol ester**

25 (19a) Using a procedure analogous to (1a), Cbz-L-2-aminobutyric acid (5.54 g, 23.4 mmol) was reacted with paraformaldehyde. Silica gel chromatography (ethyl acetate/hexanes 1:3) afforded the oxazolidinone product (5.28 g, 91%).

30 (19b) Using a procedure analogous to (1b), the material from (19a) (5.28 g, 21.2 mmol) was alkylated with allyl bromide. Silica gel chromatography (ethyl acetate/hexane, 1:9) gave the product (3.49 g, 57%). MS found (M+NH₄)⁺ = 307.

(19c) Following a procedure analogous to (1c), the material from (19b) (1.72 g, 5.88 mmol) was reacted with sodium methoxide in methanol. Silica gel chromatography gave the desired product (1.7 g, 100%). MS found $(M+H)^+ = 292$.

5 (19d) The material from (19c) (1.7 g, 5.83 mmol) was treated with sodium periodate (3.74 g, 17.5 mmol) and a 2.5 % solution of osmium tetroxide in t-butanol (0.6 mL) in a mixture of methanol (50 mL) and water (30 mL). When tlc indicated complete consumption of starting material, the reaction was diluted with water and extracted with dichloromethane. The organic phase was dried (Na_2SO_4) and
10 concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate/hexane, 1:4) to give the desired aldehyde (0.91 g, 53%). MS found $(M+H)^+ = 294$.

(19e) Following a procedure analogous to (1e), the aldehyde from (19d)
15 (0.91 g, 3.1 mmol) was reductively aminated with cyclohexylalanine methyl ester hydrochloride. The crude product was purified by silica gel chromatography to afford the desired lactam (1.0 g, 75%) as a 1:1 mixture of diastereomers. MS found $(M+H)^+ = 431$.

20 (19f) Following a procedure analogous to (1f), the lactam from (19e) (0.5 g, 1.16 mmol) was hydrogenated to afford the desired amine (0.31 g, 91%), which was used in the subsequent step without purification.

(19g) Following a procedure analogous to (1g), the amine from (19f) (0.21 g, 0.72 mmol) was coupled to *N*-(pyrazine-2-carbonyl)-L-valine. The crude product was
25 purified by silica gel chromatography (ethyl acetate/hexane, 3:1) to afford the desired peptide lactam (0.35 g, 100%).

(19h) Following a procedure analogous to (1h), the material from (19g) (0.13 g, 0.26 mmol) was saponified with lithium hydroxide monohydrate to give the desired
30 acid (0.107 g, 84%). MS found $(M+H)^+ = 488$.

(19i) Following a procedure analogous to (1i), the acid from (19h) (0.087 g, 0.18 mmol) was coupled to the (+)-pinanediol ester of L-boroallylglycine. The crude product was purified by silica gel chromatography to give the desired product as a 1:1 mixture of diastereomers. MS found $(M+H)^+ = 719$.

5

EXAMPLE 20

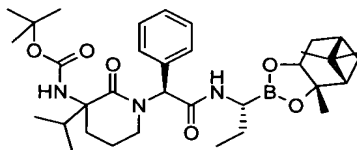
(1*R*)-1-(((2*S*)-2-(3-(((benzyloxy)carbonyl)amino)-3-isopropyl-2-oxo-1-piperidinyl)-3-cyclohexylpropanoyl)-amino}propylboronic acid (+)-pinanediol ester

10

(20a) Following a procedure analogous to (3e), the material from (2d) was coupled to L-boro-2-aminobutyric acid hydrochloride. The crude product was purified by silica gel chromatography to afford the desired boronic ester (0.034 g, 74%). MS found: $(M+H)^+ = 665$.

15

EXAMPLE 21



(1*R*)-1-(((3-((tert-butoxycarbonyl)amino)-3-isopropyl-2-oxo-1-piperidinyl)(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

20

(21a) Using a procedure analogous to (1a), Boc-L-valine (22.4 g, 103 mmol) was treated with paraformaldehyde and *p*-toluenesulfonic acid in benzene. The desired oxazolidine was obtained as a colorless solid (14.3 g, 61%).

25

(21b) Using a procedure analogous to (1b), oxazolidinone (21a) (14.3 g, 62.4 mmol) was alkylated with allyl bromide. The desired allylated oxazolidinone was obtained as a yellow oil (15.45 g, 92%). MS found $(M+Na)^+ = 292$.

(21c) A solution of 2N sodium hydroxide (10 mL) was added to oxazolidinone (21b) (2.70 g, 10 mol) in methanol (10 mL). The reaction mixture was warmed to 50°C for 5 h and then quenched with 1N hydrochloric acid (20 mL). The mixture was extracted with ethyl acetate (3x), dried (MgSO₄), and concentrated under reduced pressure. The desired acid was obtained as a yellow oil (2.6 g, 100%). MS found (M-H)⁻ = 256.

(21d) A solution of acid (21c) (2.19 g, 8.51 mmol) and benzyl bromide (0.962 mL) in acetone (50 mL) was heated to reflux in the presence of potassium carbonate (1.8 g). After 3.5 h, the reaction mixture was concentrated, resuspended in hexane, and filtered through Celite. This solution was concentrated and the residue was purified by chromatography on silica gel to afford the desired ester (2.44 g, 82%) as a colorless oil. MS found (M+Na)⁺ = 370.

(21e) Using a procedure analogous to (2a), ester (21d) (2.44g, 7.02 mmol) was hydroborated with 9-borabicyclo(3.3.1)nonane and oxidized to afford the desired alcohol (1.84 g, 72%) after chromatography on silica gel. MS found (M+Na)⁺ = 388.

(21f) Using a procedure analogous to (2b), alcohol (21e) (1.84 g, 5.03 mmol) was added to the reagent generated by addition of dimethyl sulfoxide to oxalyl chloride in dichloromethane at -78 °C. After aqueous workup, the desired aldehyde was obtained (1.99 g) and used without further purification. (M+Na)⁺ = 386.

(21g) Using a procedure analogous to (1e), the aldehyde from (21f) (0.679 g, 1.8 mmol) was reacted with L-phenylglycine methyl ester hydrochloride. Silica gel chromatography (5% acetone/toluene) afforded the desired amine as a 1:1 mixture of diastereomers (0.55 g, 62%). (M+H)⁺ = 513.

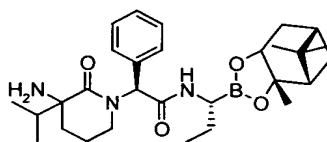
(21h) A solution of amine (21g) (389 mg, 0.76 mmol) in ethanol (10 mL) was hydrogenated over 10% palladium on carbon (43 mg) for 40 min. The solution was filtered through Celite and concentrated under reduced pressure to afford the desired acid as a white solid (315 mg, 98%). (M+H)⁺ = 423.

(21i) A solution of acid (21h) (315 mg, 0.745 mmol) and HOAt (104 mg, 0.76 mmol) in dichloromethane (10 mL) at 0 °C was treated with EDCI (157 mg, 0.82 mmol). After 20 min, the reaction mixture was warmed to room temperature and stirred for 30 min. The reaction was diluted with dichloromethane and washed with 1N hydrochloric acid. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. Chromatography on silica gel (20-30% ethyl acetate/hexane) afforded the desired lactam (0.32 g, 95%) as a 1:1 mixture of diastereomers. (M+H)⁺ = 405.

(21j) Following a procedure analogous to (1h), except that tetrahydrofuran replaced dimethoxyethane as solvent, the material from (21i) (0.200 g, 0.494 mmol) was saponified with lithium hydroxide to afford the desired acid (180 mg, 93%). (2M-H)⁻ = 779.

(21k) Using a procedure analogous to (3e), the acid from (21j) (180 mg, 0.46 mmol) was coupled to L-boro-2-aminobutyric acid hydrochloride salt. Chromatography on silica gel and HPLC afforded the desired boronic ester (99 mg, 35%). (M+H)⁺ = 610.

EXAMPLE 22

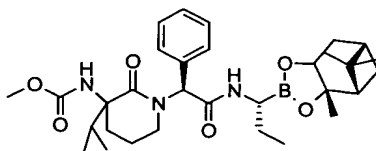


ClH

(1R)-1-(((3-amino-3-isopropyl-2-oxo-1-piperidiny)(phenyl)acetyl)amino}propylboronic acid hydrochloride (+)-pinanediol ester

(22a) The material from procedure (21k) (99 mg, 0.16 mmol) was treated with 4M hydrogen chloride solution in 1,4-dioxane (2 mL) for 5.5 h. The reaction mixture was concentrated to afford the desired amine (82 mg, 94%). (M+H)⁺ = 510.

5

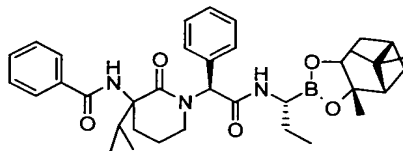
EXAMPLE 23

(1R)-1-{{{3-isopropyl-3-((methoxycarbonyl)amino)-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

10

(23a) Using a procedure analogous to (5a), the amine hydrochloride from (22a) (7 mg, 0.013 mmol) was reacted with methyl chloroformate. After purification by HPLC, the desired product was obtained (2.6 mg, 36%). (M+H)⁺ = 568.

15

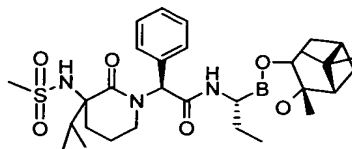
EXAMPLE 24

(1R)-1-{{{3-(benzoylamino)-3-isopropyl-2-oxo-1-piperidinyl}(phenyl)acetyl)amino}propylboronic acid (+)-pinanediol ester

20

(24a) Using a procedure analogous to (5a), the amine hydrochloride from (22a) (7 mg, 0.013 mmol) was reacted with benzoyl chloride. After purification by HPLC, the desired product was obtained (3.9 mg, 49%). (M+H)⁺ = 614.

EXAMPLE 25

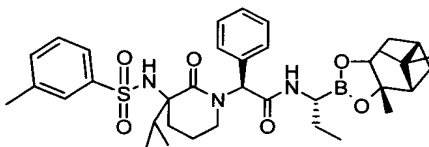


5 **(1R)-1-(((3-isopropyl-3-((methylsulfonyl)amino)-2-oxo-1-piperidinyl)(phenyl)acetyl)amino)propylboronic acid (+)-pinanediol ester**

(25a) Using a procedure analogous to (5a), the amine hydrochloride from (22a) (7 mg, 0.013 mmol) was reacted with methanesulfonyl chloride. After purification by HPLC, the desired product was obtained (2.2 mg, 29%). (M+H)⁺ = 588.

10

EXAMPLE 26



15 **(1R)-1-(((3-isopropyl-3-(((3-methylphenyl)sulfonyl)amino)-2-oxo-1-piperidinyl)(phenyl)acetyl)amino)propylboronic acid (+)-pinanediol ester**

(26a) Using a procedure analogous to (5a), the amine hydrochloride from (22a) (7 mg, 0.013 mmol) was reacted with 3-methylbenzenesulfonyl chloride. After purification by HPLC, the desired product was obtained (2.7 mg, 31%). (M+H)⁺ = 664.

20

PREPARATION OF INTERMEDIATE α -AMINOBORONIC ACIDS

Preparation of H-boroAlg-pinanediol•HCl (R=allyl)

25 Formula: H₂NCH(CH₂CH=CH₂)BO₂C₁₀H₁₆•HCl

2-Propene boronate pinanediol ester. Ether (300 mL) was placed in a 5 L, 4 neck flask equipped with two addition funnels, thermometer and a mechanical stirrer.

Triisopropyl borate (Aldrich) (1 mol) in 600 mL of anhydrous ether and allylmagnesium bromide in ether (Aldrich) (1.0 mol, 1.0 L, 1.0 M) were added simultaneously to 300 mL of dry ether at -78°C over a period of 2.5 hours. The mixture was warmed to room temperature and stirred for 12 h. The slurry was
 5 recooled to 0°C, followed by dropwise addition of 40 % sulfuric acid (2 mol) over a 1 hour period. The mixture was warmed to room temperature and was allowed to stir for 2 hours. The organic layer was separated and (+)-pinanediol (1.0 mol) was added. After 12 h, the solution was dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* and distilled (bp 85-87°C, 1 mm Hg) to give 118 g (53 %) of
 10 product as a clear, semi-viscous liquid: ¹H-NMR (CDCl₃) δ 5.8 - 6.0 (m, 1H), 4.9 - 5.1 (m, 2H), 4.2 (dd, 1H), 2.8 (m, 2H), 2.05-1.78 (m, 6H), 1.38 (s, 3H), 1.27 (s, 3H), 0.83 (s, 3H).

1-Chloro-3-butene boronate pinanediol ester. The α-chloro compound was prepared by homologation of the corresponding allyl boronate. To a 5-liter flask
 15 equipped with two addition funnels, thermometer and a mechanical stirrer, was added the allyl boronate (117, 0.53 mol) dissolved in dry THF (1 L), followed by the addition of cyclohexane (0.5 L) and dichloromethane (0.71 mol). The solution was cooled to -78°C, followed by dropwise addition of lithium diisopropylamide (LDA) in heptane/ THF/ ethylbenzene (0.64 mol, 2.0 M, Aldrich catalog number 36,179-8) over
 20 a 1 hour period, taking care that a reaction temperature between -60 to -78°C was maintained. Anhydrous zinc chloride in ether (0.86 mol, 1.0 M) was added. The reaction was warmed to room temperature and stirred for 12 hours. Hexane (600 mL) was added and the mixture was stirred for 1 hour. Cold 1 N H₂SO₄ (3.2 L) was added and the phases were separated. The aqueous layer was washed with hexane (600 mL).
 25 The combined organic phases were concentrated to 1 L and washed with 5% sodium bicarbonate (1 L) and saturated sodium chloride (1 L). They were dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* and distilled (bp 130-132°C, 0.5 mm Hg) to give 60 g (42 %) of the α-chloroboronic acid as a clear yellow oil. ¹H-NMR (CDCl₃) δ 5.8 - 6.0 (m, 1H), 5.2 (m, 2H), 4.2 (dd, 1H), 3.48 (q, 1H)
 30 2.8 (m, 2H), 2.05-1.78 (m, 6H), 1.41 (s, 3H), 1.29 (s, 3H), 0.84 (s, 3H).

H-boroAlg pinanediol ester·hydrochloride. The *bis*-trimethylsilane protected amine (Scheme 8) was prepared by dissolving hexamethyldisilazane (64.4 mmol) in

dry THF (30 mL) and cooling to -78°C . *n*-Butyl lithium in hexane (1.6 N, 70.8 mmol) was added and the solution was allowed to warm to room temperature. It was recooled to -78°C and 1-chloro-3-butene boronate pinanediol (17.2 g, 64.4 mmol) was added in 30 mL THF. The mixture was allowed to slowly warm to room temperature and to stir overnight. Solvent was removed by evaporation and dry hexane (200 mL) was added. Insoluble material was removed by filtration under a nitrogen atmosphere through a bed of celite to yield a solution of the protected amine. This solution was cooled to -78°C and 4 N anhydrous hydrogen chloride in dioxane (192 mmol) was added. The reaction was slowly allowed to warm to room temperature and to stir overnight. The solvent was evaporated under vacuum to yield a brown oil. It was purified on a 5 x 90 cm column of SephadexTM LH-20 in methanol. TLC in ethyl acetate:hexane (1:1) indicated the product as a single base spot which gave a positive test for amines after spraying with ninhydrin. The product eluted in fractions 51-70 (10mL fractions). The fractions were pooled, concentrated, and dried under vacuum to give 16 g (87.2%) of the desired product as a foam. $^1\text{H-NMR}$ (CDCl_3) δ 8.21 (bs, 2H), 5.80 - 6.0 (m, 1H), 5.20 (m, 2H), 4.2 (dd, 1H), 3.0 (m, 1H), 2.62 (m, 2H), 2.4 - 1.78 (m, 6H), 1.41 (s, 3H), 1.29 (s, 3H), 0.80 (s, 3H).

Preparation of boroAbu-pinanediol ester (R=ethyl)

Formula: $\text{H}_2\text{NCH}(\text{CH}_2\text{CH}_3)\text{BO}_2\text{C}_{10}\text{H}_{16}\bullet\text{HCl}$

Propane boronate pinanediol ester. The alkyl boronate was prepared on a 0.50 mole scale using a procedure similar to the one used in the preparation of allyl boronate pinanediol. The crude product was distilled (bp 63°C , 2 mm Hg) to give 32.3 g (41.4 %) as a clear oil. $^1\text{H-NMR}$ (CDCl_3) δ 4.23 (dd, 1H), 2.40-1.78 (m, 6H), 1.38 (s, 3H), 1.28 (s, 3H), 0.97 (t, 3H), 0.83 (s, 3H), 0.79 (q, 2H).

1-Chloropropane boronate pinanediol ester. The α -chloro boronic acid was prepared on a 0.21 mole scale by the procedure described for H-boroAlg-pinanediol except the reaction mixture was washed with saturated aqueous ammonium chloride (1000 mL) rather than sulfuric acid. Phases were separated and the aqueous layer was washed with an equal volume of hexane. The organic phases were combined, dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product which was distilled (bp 100 - 102°C , 0.6 mm Hg) to yield 28.8g (54.4 %) of the desired

product as a clear yellow oil. $^1\text{H-NMR}$ (CDCl_3) δ 4.35 (dd, 1H), 3.41 (m, 1H), 2.40-1.80 (m, 8H), 1.41 (s, 3H), 1.29 (s, 3H), 1.02 (t, 3H), 0.84 (s, 3H).

H-boroAbu pinanediol ester•hydrochloride. The amino boronic acid was prepared on a 0.09 mole scale and was purified by a procedure similar to the one described for Example 1 to yield 23 g of crude product. A proportion of this material (13 g) was purified by chromatography on an LH-20 column to give 7.47 g (54.9 %) of the desired product as a brown foam. $^1\text{H-NMR}$ (CDCl_3) δ 8.24 (s, 3H), 4.36 (dd, 1H), 2.91 (m, 1H), 1.8-2.4 (m, 8H), 1.41 (s, 3H), 1.27 (s, 3H), 1.08 (t, 3H), 0.82 (s, 3H).

Preparation of boro-Cyclopropylglycine pinacol ester (R= cyclopropyl)

Formula: $\text{H}_2\text{NCH}(\text{C}_3\text{H}_5)\text{BO}_2\text{C}_{10}\text{H}_{16}\cdot\text{HCl}$

Cyclopropylboronate pinacol ester. The pinacol cyclopropyl boronate ester was prepared by the addition of cyclopropyl magnesium bromide to isopropylboronate pinacol ester. The latter compound was prepared by a previously described procedure (Andersen, M. W.; Hildebrandt, B.; Koster, G.; Hoffmann, R. W. *Chem. Ber.* **122**, 1989, 1777-1782). The Grignard reagent was prepared by adding cyclopropylbromide (3.0 mL, 37 mmol) to magnesium turnings (11 g, 0.46 mole) in THF (300 mL) at room temperature under nitrogen. The solution was carefully warmed to 42°C at which point a vigorous exotherm ensued. After the exotherm had subsided an additional 3 mL of cyclopropylbromide was added and an exotherm ensued and subsided. This iterative process was repeated until all of the cyclopropyl bromide was added (36 mL, 0.45 mole). The solution was heated at 50°C for an additional 2 h. At this time the contents of the flask were transferred to an addition funnel and added to a solution of isopropylboronate pinacol ester (84 g, 0.45 mol) in ether (400 mL) in a 3-necked, 2-liter flask in ether (500 mL) at -78°C under nitrogen. The cyclopropyl Grignard reagent was added dropwise over a period of 3 h. The solution was allowed to warm to room temperature and stirred overnight. The solution was cooled to 0°C and 1 N HCl prepared in saturated aqueous NaCl (500 mL) was added dropwise over a period of 1 h. The solution was allowed to stir for an additional 4 h and the layers were separated. The aqueous layer was extracted with hexanes (3 x 300 mL), dried over MgSO_4 , and concentrated using a rotary evaporator.

The residue was purified by silica gel chromatography using 10% ethyl acetate: hexanes as a solvent to yield a clear colorless oil (42 g, 0.25 mole, 56%), bp 50-52°C, 8 mm Hg. ¹H NMR d 0.36-0.50 (m, 5H), 1.18 (s, 12H).

- 1-Chloro-1-cyclopropylmethyl boronate pinacol ester. A 3-necked 250 mL flask containing THF (75 mL) and dichloromethane (2.5 mL, 39 mmol) was cooled to -100°C. *n*-Butyllithium (1.6 M in Hexanes, 24 mL, 39 mmol) was added cautiously to maintain a solution temperature of -100°C. After stirring at -100°C for 45 min, a solution of cyclopropylboronate pinacol ester (6.0 g, 36 mmol) in THF (10 mL) precooled to -78°C was added. The solution was allowed to warm to room temperature and stirred for an additional 12 h. The solution was concentrated by evaporation and hexanes were added to give a solid. The mixture was filtered and the filtrate was evaporated to give an oil. This material was distilled through a short path distillation apparatus (67-70°C, 0.2 mm Hg) to yield a clear colorless oil (5.5 g, 58 % yield). ¹H-NMR d (CDCl₃) 2.87 (d, 2H), 1.27 (s, 12H), 0.63 (m, 3H), 0.37 (m, 2H).
- H-boroCyclopropylglycine pinanediol ester. The α-chloro compound (5.0 g, 23 mmol) was dissolved in THF (50 mL) and added to a freshly prepared solution of lithium *bis*-trimethylsilylamide (100 mL of a 3.2 M solution) at -78°C under nitrogen. The solution was warmed to room temperature and stirred for 18 h. THF was removed by rotary evaporation and hexanes were added to the oil to give a precipitate. The solid was removed by filtration and the filtrate was cooled to -78°C. A solution of 4 N HCl in dioxane (17 mL, 69 mmol, 3 equivalents) was added and the solution was stirred for 4 h while warming to room temperature to yield a solid. It was isolated by filtration and dissolved in hot CHCl₃ (150 mL). Following concentration to 10 mL, hot ethyl acetate (~25 mL) was added. Slow crystallization gave the desired product (3.3 g, 14 mmol, 60% yield). ¹H NMR (CDCl₃) 8.22 (br. s, 3H), 3.47 (m, 1H), 1.28 (s, 12H), 0.65 (m, 4H), 0.38 (m, 1H).

Preparation of H-borodifluoroethylglycine pinanediol(R= 2,2-difluoroethyl)

Formula: H₂NCH(CH₂CHF₂)BO₂C₁₀H₁₆•HCl

- Chloromethyl boronate pinacol ester. Tetrahydrofuran (150 mL) was placed in a 1 L, 3 neck flask equipped with two addition funnels. Triisopropyl borate (Aldrich) (32.1 mL, 139 mmol) and chloro-iodomethane (Aldrich) (10.3 mL, 142

mmol) were added to the flask. The reaction mixture was cooled to -78°C . *n*-Butyllithium (81.9 mL, 131 mmol, 1.6 M in hexanes) was added dropwise to the flask via an addition funnel. The solution was stirred at -78°C for 2 hours and then gradually warmed to -10°C . A crystal of methyl orange was added to the reaction.

- 5 Hydrogen chloride (1.0 N in ether) was added via the other addition funnel until the methyl orange end point was reached. Pinacol (16.4g, 139 mmol) was added to the flask and the reaction mixture was stirred for 12 hours. It was then concentrated *in vacuo* and distilled (bp $61\text{--}63^{\circ}\text{C}$, 5 mm Hg) to give 16.0 g (65 %) of the desired compound as a yellow oil. ^1H NMR (CDCl_3) δ 2.97 (s, 2H, ClCH_2B), 1.29 (s, 12H, CCH₃).

- Iodomethyl boronate pinacol. THF (800 mL) was placed in a 3 L, 3-necked flask equipped with two addition funnels. Triisopropyl boronate (Aldrich) (128 mL, 0.55 mol) and chloro-iodomethane (Aldrich) (100 g, 0.56 mol) were added. The mixture was cooled to -78°C and *n* butyl lithium (330 mL, 0.53 mol, 1.6 M in
- 15 hexanes) was added dropwise. The solution was stirred for 2h and slowly allowed to warm to -10°C . Methyl orange indicator was added and HCl (1.0 M in ether) was added until the methyl orange endpoint was reached. Pinacol (65 g, 0.55 mol) was added and reaction mixture was allowed to stir 12 h. It was filtered and evaporated in *vacuo*. The residue was dissolved in acetone (500 mL) and sodium iodide (70 g, 0.47
- 20 mol) was added. After stirring for 12 h at room temperature, solvent was removed by evaporation and the residue was dissolved in ethyl acetate and washed with saturated aqueous NaCl. The organic phase was dried over Na_2SO_4 , filtered, and concentrated in *vacuo*. It was distilled to give 69 g (47%) of the desired product (bp $45\text{--}50^{\circ}\text{C}$, 1.5 mm). ^1H NMR (CDCl_3) δ 2.16 (s, 2H), 1.26 (s, 12H).

- 25 Phenylthiomethane boronate pinacol ester. Thiophenol (11.6 mL, 113 mmol) was dissolved in DMF (40 mL) and diisopropylethylamine (19.8 mL, 113 mmol) and chloromethyl boronate pinacol ester (20 g, 113 mmol) were added sequentially. (Iodomethyl boronate pinacol can be readily substituted for the chloro compound.) After stirring for 12 hours, solvent was removed by rotary evaporation and ether (70
- 30 mL) was added. The reaction mixture was washed with 0.2 N HCl (70 mL), 5 % NaHCO_3 (70 mL) and saturated sodium chloride (70 mL). The combined organic phases were dried over sodium sulfate and filtered. The filtrate was concentrated *in*

vacuo and distilled (bp 125-127°C, 0.6 mm Hg) to give 21.6 g (76 %) of the desired product as a clear oil. ¹H NMR (CDCl₃) δ 7.32 - 7.11 (m, 5H), 2.42 (s, 2H), 1.24 (s, 12H).

1-Phenylthio-3,3-difluoropropane-1-boronate pinacol ester. Butyllithium (50.6 mL, 126 mmol, 2.5 M in hexanes) was added dropwise to a solution of diisopropylamine (18.4 mL, 133 mmol) dissolved in THF (40 mL) at 0°C in a 500 mL round bottom flask. A solution of phenylthiomethane boronate pinacol ester (31.6 g, 126 mmol) in THF (40 mL) was added dropwise over a period of approximately 2 min to yield a white precipitate. After stirring for 1 hour at 0°C, 1,1-difluoro-2-bromoethane (Lancaster) (51 mL, 630 mmol) was added dropwise. The precipitate dissolved and the solution was allowed to warm to room temperature and stirred for 16 hours. Excess cold 10 % phosphoric acid was added and the mixture was stirred for 5 min. Ether (100 mL) was added and the phases were separated. The organic layer was dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* and distilled (bp 119-122°C, 0.4 mm Hg) to give 22 g (56 %) of product as a clear oil. ¹H NMR (CDCl₃) δ 7.43 - 7.19 (m, 5H, C₆H₅), 6.16 - 5.78 (tt, 1H, CHF₂), 2.82 (m, 1H, SCHB), 2.38 - 2.19 (m, 2H, CH₂CHF₂), 1.23 (s, 12H, CCH₃). ¹⁹F NMR δ -116.8 to -117.0 (dt, CHF₂).

1-Iodo-3,3-difluoropropane-1-boronate pinacol ester. 1-Phenylthio-3,3-difluoropropane-1-boronate pinacol ester (6.00 g, 19.1 mmol) was dissolved in anhydrous acetonitrile (60 mL) and dry methyl iodide (24 mL, 380 mmol) and sodium iodide (5.76 g, 38.2 mmol) were added. The reaction mixture was vigorously refluxed for 5 h. The solvent was evaporated *in vacuo*. The residue was partitioned between water (40 mL) and ether (40 mL). The phases were separated and the organic phase was washed with an equal volume of ether. The combined organic phases were dried over Na₂SO₄ and evaporated to give a brown oil which was purified by distillation to give 3.1 g (49%), bp 63-65°C, 0.4 mm. ¹H NMR (CDCl₃) δ 6.18 - 5.79 (tt, 1H, CHF₂), 3.21 (t, 1H, ICHB), 2.43 - 2.21 (m, 2H, CH₂CHF₂), 1.27 (s, 12H, CCH₃).

1-Amino-3,3-difluoropropyl boronate pinacol•HCl. 1-Iodo-3,3-difluoropropyl boronate pinacol (2.7 g, 8.1 mmol) was dissolved in THF (10 mL)

and was added dropwise to a solution of lithium bis(trimethylsilyl)amide (9.68 mL, 9.68 mmol, 1.0 M in THF) dissolved in anhydrous THF (10 mL) and cooled to -78°C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. It was concentrated *in vacuo* and hexane was added. The reaction mixture was cooled to -78°C, followed by the dropwise addition of 4 N anhydrous hydrogen chloride in dioxane (6.05 mL, 24.2 mmol). The mixture was allowed to warm to room temperature and stirred for 5 hours. The reaction mixture was evaporated and chloroform was added. Insoluble material was removed by filtration. The filtrate was evaporated almost to dryness and hexanes were added. Upon standing the product crystallized. It was isolated and washed with cold hexane to yield 1.1 g (52 %), mp 138-141°C. ¹H NMR (CDCl₃) δ 7.68 (bs, 3H), 6.22 - 6.01 (tt, 1H), 3.42 (m, 1H), 2.76 - 2.51 (m, 2H), 1.32 (s, 12H). ¹⁹F NMR δ -115.2 to -115.5 (dt, CHF₂). HRMS calculated for C₉H₁₈B₁O₂F₂N +H: 222.1. Found: 222.1.

15 **Preparation of boroVinylglycine pinanediol**

Formula: H₂NCH(CH=CH₂)BO₂C₁₀H₁₆•HCl

1-Chloro-1-vinylmethyl boronate pinanediol. The α-chlorovinyl compound was prepared by the method described by Matteson, D.S. & Majumdar, D. *Organometallics* 2, 1529-1535, 1983.

20 boro-Vinylglycine pinanediol Ester•HCl. The α-chlorovinyl boronate pinanediol ester (10.6 g, 41.7 mmol) was dissolved in THF (100 mL) and added to a freshly prepared solution of lithium hexamethyldisilazide (45.9 mmol) in THF (150 mL) at -78°C. This solution was stirred for 20 h while warming to room temperature. THF was removed *in vacuo* and hexanes (150 mL) were added. The resulting precipitate was removed by filtration. The filtrate was cooled to -78°C and a solution of HCl in dioxane (4.0 N, 31.3 mL, 125 mmol) was added. The solution was allowed to warm to room temperature and to stir for 20 h. The solvents were removed in vacuo to yield 7.2 g (26 mmol, 63 % yield) of a bright orange, viscous oil which formed a glass when placed under high vacuum. ¹H-NMR (CDCl₃) δ 0.76 (s, 3H), 1.21 (s, 3H), 1.36 (s, 3H), 1.83-2.25 (m, 6H), 3.64 (d, 2H), 4.34 (d, 1H), 5.24 (d, 1H), 5.45 (d, 1H), 5.97 (m, 1H), 8.47 (br. s, 3H).

Preparation of H-boroThreonine(OBzl)-pinanediol

Formula: $\text{H}_2\text{NCH}(\text{CH}(\text{Obenzyl})\text{CH}_3)\text{BO}_2\text{C}_{10}\text{H}_{16}\bullet\text{HCl}$

Pinacol (1-chloroethyl)boronate. A 250 mL round bottom flask is charged with THF (60 mL) and CH_2Cl_2 (2.63 mL, 41.0 mmol). The solution was cooled to -100°C with a liquid nitrogen/methanol/ H_2O bath. *n*-BuLi (1.6 N in hexanes, 25.7 mL) was added slowly over the course of 1 h. The resulting solution was stirred for an additional 45 min at -100°C. Pinacol methyl boronate, dissolved in THF (40 mL), was added and the solution was stirred overnight while warming to room temperature. The THF was removed by evaporation and hexanes (100 mL) were added. The resulting precipitate was filtered and the solution concentrated. The residue was distilled at 70°C, 2 mm Hg to yield 2.06 g (30 %) of a clear colorless oil. ^1H -NMR (CDCl_3) δ 3.49 (q, 1H), 1.52 (d, 4H), 1.27 (s, 12H).

Pinanediol (1-benzyloxyethyl)boronate. *n*-BuLi (1.6 N, 13.8 mL) was added to a solution of benzyl alcohol (2.3 mL, 22 mmol) in THF (60 mL) at -78 °C followed by DMSO (1.6 mL, 22 mmol). The solution was allowed to warm to room temperature and stir for 1 h. The solution was recooled to 0°C and a solution of Pinacol (1-chloroethyl)boronate (2.06 g, 11 mmol) in THF (60 mL) was added. The solution was stirred at room temperature for 1 h and then heated at 60°C for 5 h. The contents of the flask are poured into 0.2 N HCl (300 mL). The layers were separated and the aqueous layer was washed with ether (3 x 100 mL). The combined organic layers were washed with brine and dried over Na_2SO_4 . To this solution was added (s)-pinanediol (1.87 g, 11.0 mmol) and the solution was stirred for 1 day and concentrated to yield an oil. This oil was purified by silica gel column chromatography using 10% ethyl acetate/90% hexane as an eluent. The appropriate fractions are pooled and the solvent evaporated to yield 2.66 g (77% yield) of a pale yellow oil. ^1H -NMR (CDCl_3) δ 7.30 (m, 5H), 4.57 (s, 2H), 4.32 (d, 1H), 3.45 (dq, 1H), 2.39-1.82 (m, 6H), 1.41 (s, 3H), 1.40 (dd, 3H), 1.29 (s, 3H), 0.84 (s, 3H).

Pinanediol (2-benzyloxy-1-chloropropyl)boronate. CH_2Cl_2 (0.80 mL, 12.7 mmol) was added to THF (40 mL) and cooled to -100°C. *n*-BuLi (1.6 N, 6.3 mL) was slowly added while maintaining a temperature of -100°C. The flask was stirred at -100°C for an additional 45 min. Pinanediol (1-benzyloxyethyl)boronate (2.66 g, 8.46

mmol), dissolved in THF (20 mL), was added followed by a solution of zinc(II) chloride in ether (1.0 N, 17 mL). The THF was evaporated and the residue was redissolved in hexanes (150 mL). The solution was washed with saturated aqueous ammonium chloride, brine, and dried over MgSO_4 . It was concentrated to give a light
 5 oil. This oil was purified by silica gel column chromatography (10% ethyl acetate/90% hexanes eluant) to yield 1.55 g (51%) of a clear oil. $^1\text{H-NMR}$ (CDCl_3) δ 7.36 (m, 5H), 4.58 (m, 2H), 4.37 (d, 1H), 3.91 (m, 1H), 3.56 (d, 2H), 2.39-1.81 (m, 6H), 1.40 (d, 3H), 1.34 (d, 3H), 1.29 (s, 3H), 0.84 (s, 3H).

Pinanediol (2-benzyloxy-1-aminopropyl)boronate•HCl. Pinanediol (2-
 10 benzyloxy-1-chloropropyl)boronate, dissolved (3.85 g, 10.6 mmol) in THF (60 mL), was added to a solution of LiHMDS (10.6 mmol) in THF at -78°C . The solution was stirred for 1 h at -78°C and allowed to warm to room temperature. Solvent was evaporated and the residue redissolved in hexanes (120 mL). The solid was filtered and the filtrate recooled to -78°C , and a solution of HCl in 1,4-dioxane (4 N, 8.0 mL)
 15 was added. The solution was allowed to warm to room temperature while stirring overnight. The solvent was evaporated to yield 2.55 g (63%) of a brown oil. $^1\text{H-NMR}$ (CDCl_3) δ 8.11 (br s, 3H), 7.35 (m, 5H), 4.57 (m, 2H), 4.32 (m, 1H), 3.16 (br s, 1H), 2.34-1.83 (m, 6H), 1.38 (s, 3H), 1.33 (m, 3H), 1.24 (s, 3H), 0.79 (s, 3H).

20 **Preparation of H-boroSer(OBzl)-pinanediol HCl**

Formula: $\text{H}_2\text{NCH}(\text{CH}_2\text{Obenzyl})\text{BO}_2\text{C}_{10}\text{H}_{16}\bullet\text{HCl}$

H-boroSer(OBzl)-pinanediol HCl was prepared by adding Pinanediol 1-chloro-2-benzyloxy-boronate (5.0g, 14.3 mmol) in THF (60 mL) to a solution of LiHMDS (15 mmol) in THF (60 mL) at -78°C . The solution was allowed to stir
 25 while warming to room temperature over a period of 3 h. The THF was evaporated, the residue redissolved in anhydrous hexanes (200 mL), cooled to -78°C , and a solution of HCl in dioxane (4 N, 11.3 mL) was added. The resulting mixture was allowed to stir while warming to room temperature. The solids were removed by filtration. The filtrate was evaporated and triturated with chloroform (50 mL) and
 30 refiltered. The chloroform was evaporated and the residue dissolved in hot hexanes (30 mL). As the hexanes were allowed to cool a cream colored solid crystallized. This solid was combined with a solid that had crystallized from the original hexanes

filtrate. The combined solids were filtered, dried in vacuo to yield 2.4 g (46%) of a cream colored solid, mp 112-115°C. ¹H-NMR (CDCl₃) 8.16 (br s., 3H), 4.59 (dd, 2H), 4.37 (d, 1H), 4.02 (m, 1H), 3.83 (m, 1H), 3.31 (br s, 1H), 2.31-2.11 (m, 2H), 2.02 (t, 1H), 1.91-1.84 (m, 3H), 1.39 (s, 3H), 1.25 (s, 3H), 0.79 (s, 3H). MS/ESI
 5 calculated for C₁₉H₂₉BNO₃ + H⁺: 330.2: Found: 330.3.

Preparation of Pinanediol 1-amino-2-thiophenylethylboronate HCl

Formula: H₂NCH(CH₂SC₆H₅)BO₂C₁₀H₁₆•HCl

Pinanediol 1-chloro-2-thio(phenyl)ethylboronate. Phenylsulfenyl chloride
 10 (2.0 g, 13.8 mmol) was added to a solution of pinanediol vinyl boronate (2.85 g, 13.8 mmol) in CH₂Cl₂ (30 mL). The solution was stirred for 30 min and then the solution was evaporated to yield 3.9 g (81%) of a pale yellow oil. ¹H-NMR (CDCl₃) δ 7.40 (m, 5H), 4.40 (d, 1H), 3.49 (m, 1H), 3.64 (m, 1H), 3.33 (m, 2H), 2.34-1.89 (m, 6H), 1.43 (s, 3H), 1.30 (s, 3H), 0.85 (s, 3H). MS/APCI calculated for C₁₈H₂₄BClO₄S + H:
 15 351.1. Found: 351.0.

Pinanediol 1-amino-2-thiophenylethylboronate HCl. Pinanediol 1-chloro-2-thio(phenyl)ethylboronate (2.0 g, 5.7 mmol) dissolved in THF (40 mL) was added to a solution LiHMDS (6.0 mmol) in THF (60 mL) at -78°C. The solution was allowed to warm to room temperature and solvent was evaporated. The residue was redissolved
 20 in hexanes, filtered and recooled to -78°C. A solution of HCl in dioxane (4 N, 5 mL) was added and the mixture was allowed to stir overnight while warming to room temperature. The solvent was removed to yield 1.2 g (57%) of the desired product as a yellow foam. ¹H-NMR δ 8.46 (br s, 3H), 4.33 (d, 1H), 3.75 (s, 3H), 3.48 (br s, 2H), 3.15 (m, H), 2.4-1.8 (m, 6H), 1.35 (s, 3H), 1.23 (s, 3H), 0.78 (s, 3H). MS/ESI
 25 calculated for C₁₈H₂₇BNO₂S: 332.3. Found: 332.2.

Pinanediol 1-amino-2-thiolsulfenyl(phenyl)ethyl boronate

Formula: H₂NCH(CH₂SSC₆H₅)BO₂C₁₀H₁₆•HCl

1-Chloro-2-thiolsulfenyl(phenyl)ethyl boronate pinanediol.
 30 Phenylthiosulfenyl chloride was prepared by reacting benzene thiol with sulfur dichloride at -78°C using a published procedure (Can. J. Chem., 51, 3403-3412,

1973). 1-Chloro-2-thiolsulfenyl(phenyl)ethyl boronate pinanediol was obtained by adding phenylthiosulfenyl chloride (3.2 g, 18.2 mmol) dissolved in dichloromethane (30 mL) dropwise over a period of 10 min to a solution of pinanediol vinylboronate (3.7 g, 18.2 mmol) in CH₂Cl₂ (50 mL) in the presence of CaCO₃ (30 mg). The
 5 resulting solution was stirred for an additional 1 h at room temperature. The contents of the flask were poured into brine (100 mL), the layers were separated and the organic layer was dried over Na₂SO₄. The organic layer was evaporated to yield a pale, yellow-green oil which was further purified by silica gel column chromatography (eluant 1% EtOAc/99% Hexanes). The appropriate fractions were
 10 pooled and evaporated to yield 2.93 g (7.8 mmol, 43%) of a pale green viscous oil. MS/APCI calculated for C₁₈H₂₄BClO₂S₂ + H: 383. Found: 383. ¹H-NMR CDCl₃ δ 0.85 (s, 3H), 1.30 (s, 3H), 1.42 (s, 3H), 1.86-2.40 (m, 6H), 3.11-3.32 (m, 2H), 3.73 (t, 1H), 4.37 (dd, 1H), 7.22-7.63 (m, 5H).

Pinanediol 1-amino-2-thiolsulfenyl(phenyl)ethyl boronate. 1-Chloro-2-
 15 thiolsulfenyl(phenyl)ethyl boronate pinanediol was treated with lithium hexamethyldisilane by the procedure in pinanediol 1-amino-2-thiophenylethylboronate to yield the alpha-amino compound. MS/ESI calculated for C₁₈H₂₆BNO₂S₂ + H: 364. Found: 364.

20 **Preparation of Pinacol 1-amino-3,3,3-trifluorobutyl boronate**

Formula: H₂NCH(CH₂CH₂CF₃)BO₂C₁₀H₁₆•HCl

1-Phenylthio-4,4,4-trifluorobutane-1-boronate pinacol ester.

Phenylthiomethane boronate pinacol ester was prepared by the procedure in H-borodifluoroethylglycine pinanediol. Diisopropylamine (4.7 mL, 33.6 mmol) was
 25 dissolved in THF (10 mL) and stirred at 0 °C in a 100 mL round bottom flask. Butyllithium (12.8 mL, 32.0 mmol, 2.5M in hexanes) was added dropwise to the solution. A solution of phenylthiomethane boronate pinacol ester (8.0 g, 32.0 mmol) in THF (10 mL) was added dropwise rapidly, yielding a white precipitate. The reaction mixture was stirred for 1 hour at 0 °C, followed by the dropwise addition of
 30 3,3,3-trifluoropropyl iodide (Lancaster) (15.0g, 64.0 mmol). The precipitate dissolved and the solution was allowed to warm to room temperature and stirred for 12 hours. The mixture was then treated with excess cold 10 % phosphoric acid and

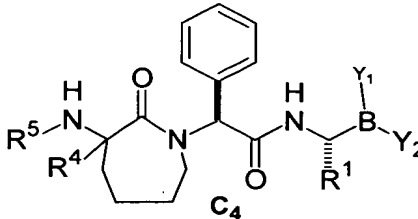
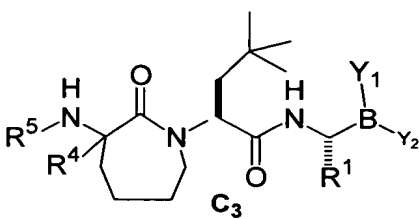
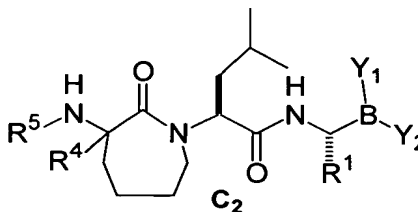
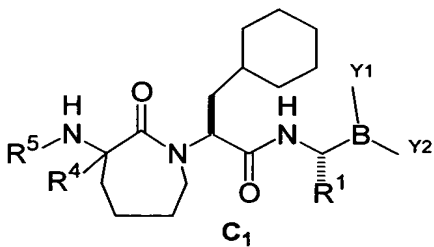
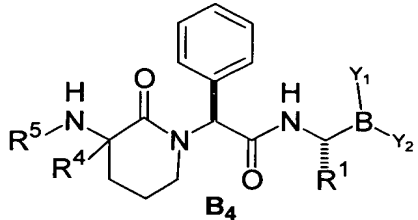
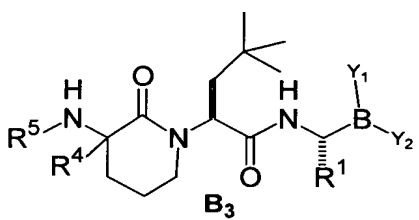
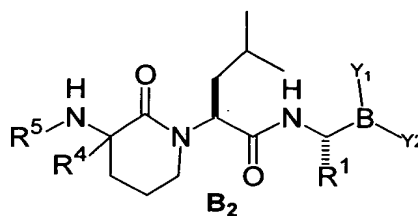
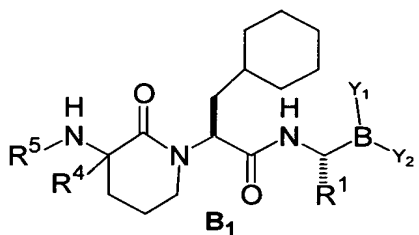
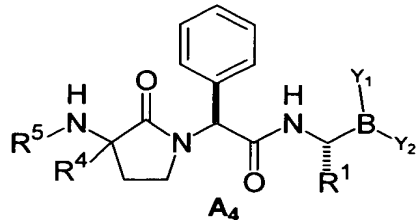
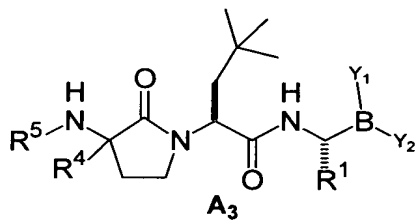
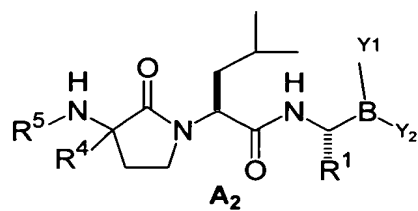
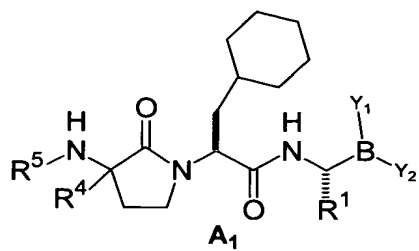
stirred for 5 minutes. The reaction mixture was poured into a separatory funnel and extracted with ether (100 mL). The organic layer was dried over sodium sulfate and filtered. The filtrate was concentrated *in vacuo* and distilled (bp 112-114 °C, 0.25 mm Hg) to give 6.53g (59 %) of the desired product as a clear oil. ¹H nmr (CDCl₃) δ 7.41 - 7.11 (m, 5H, C₆H₅), 2.78 (t, 1H, SCHB), 2.35 (m, 2H, CH₂CF₃), 1.98 (m, 1H, CH₂CH₂CF₃), 1.23 (s, 12H, CCH₃). ¹⁹F nmr δ -116.8 to -117.0 (t, 3H, CF₃).

1-iodo-4,4,4-trifluorobutane-1-boronate pinacol ester. 1-Phenylthio-4,4,4-trifluorobutane-1-boronate pinacol ester (3.3g, 9.5 mmol) was dissolved in anhydrous acetonitrile (33 mL). Dry methyl iodide (11.9 mL, 190.6 mmol) was added, followed by the addition of sodium iodide (2.87g, 19.1 mmol). The reaction mixture was refluxed for 12 h. The solvent was evaporated to give an oily residue which was purified by distillation to give 3.32g (95.6 %), bp 51 °C, 0.5 mm Hg. ¹H nmr (CDCl₃) δ 3.21 (t, 1H, ICHB), 2.39 (m, 2H, CH₂CF₃), 2.05 (m, 2H, CH₂CH₂CF₃), 1.27(s, 12H, CCH₃).

1-amino-4,4,4-trifluorobutyl boronate pinanediol ester. 1-iodo-4,4,4-trifluorobutyl pinacol ester (3.4g, 9.58 mmol) was dissolved in THF (20 mL) and was added dropwise to a solution of lithium bis(trimethylsilyl)amide (Aldrich) (9.6 ml, 9.6 mmol, 1.0M in THF) dissolved in anhydrous THF (20 ml and cooled to -78 °C). The reaction mixture was allowed to warm to room temperature and stirred for 12 hours. It was concentrated *in vacuo* and hexane was added. The reaction mixture was cooled to -78 °C and 4M anhydrous hydrogen chloride in dioxane (7.2 ml, 28.7 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was concentrated and chloroform was added. Insoluble material was removed by filtration. The filtrate was evaporated almost to dryness and hexanes were added. Upon standing the product crystallized. It was isolated and washed with cold hexanes to yield 1.7g (69.8 %) of a brown solid. ¹H nmr (CDCl₃) δ 7.80 (bs, 3H), 3.19 (m, 1H), 2.78 (m, 1H), 2.58 – 2.05 (m, 3H), 1.23 (s, 12H). ¹⁹F nmr (CDCl₃) δ -66.67 to -66.59 (t, 3H, CF₃).

The following table contains representative examples envisioned by the present invention. For each compound, both epimers at the quaternary center (bearing the R⁴ substituent) are considered to be specified in the table.

TABLE 2



5

R ¹	R ⁴	R ⁵
ethyl	ethyl	m-methylphenylsulfonyl
ethyl	ethyl	m-trifluoromethyl-phenylsulfonyl
ethyl	ethyl	p-isopropylphenyl-sulfonyl
ethyl	ethyl	p-propylphenylsulfonyl
ethyl	ethyl	p-t-butylphenylsulfonyl
ethyl	ethyl	p-carboxylphenyl-sulfonyl
ethyl	ethyl	4-biphenylsulfonyl
ethyl	ethyl	1-naphthylsulfonyl
ethyl	ethyl	2-naphthylsulfonyl
ethyl	ethyl	8-quinolinesulfonyl
ethyl	ethyl	benzyl
ethyl	ethyl	N-phenylcarbamoyl
ethyl	ethyl	N-(p-butylphenyl)carbamoyl
ethyl	ethyl	butylsulfonyl
ethyl	ethyl	carbobenzyloxy
ethyl	ethyl	methoxycarbonyl
ethyl	ethyl	benzoyl
ethyl	ethyl	methanesulfonyl
ethyl	ethyl	phenylsulfonyl
ethyl	ethyl	o-nitrophenylsulfonyl
ethyl	ethyl	m-nitrophenylsulfonyl
ethyl	ethyl	m-aminophenylsulfonyl
ethyl	propyl	m-methylphenylsulfonyl
ethyl	propyl	m-trifluoromethyl-phenylsulfonyl
ethyl	propyl	p-isopropylphenyl-sulfonyl
ethyl	propyl	p-propylphenylsulfonyl
ethyl	propyl	p-t-butylphenylsulfonyl
ethyl	propyl	p-carboxylphenyl-sulfonyl
ethyl	propyl	4-biphenylsulfonyl
ethyl	propyl	1-naphthylsulfonyl
ethyl	propyl	2-naphthylsulfonyl
ethyl	propyl	8-quinolinesulfonyl
ethyl	propyl	benzyl
ethyl	propyl	N-phenylcarbamoyl
ethyl	propyl	N-(p-butylphenyl)carbamoyl
ethyl	propyl	butylsulfonyl
ethyl	propyl	carbobenzyloxy
ethyl	propyl	methoxycarbonyl
ethyl	propyl	benzoyl
ethyl	propyl	methanesulfonyl
ethyl	propyl	phenylsulfonyl
ethyl	propyl	o-nitrophenylsulfonyl
ethyl	propyl	m-nitrophenylsulfonyl
ethyl	propyl	m-aminophenylsulfonyl
ethyl	isopropyl	m-methylphenylsulfonyl

R ¹	R ⁴	R ⁵
ethyl	isopropyl	m-trifluoromethyl-phenylsulfonyl
ethyl	isopropyl	p-isopropylphenyl-sulfonyl
ethyl	isopropyl	p-propylphenylsulfonyl
ethyl	isopropyl	p-t-butylphenylsulfonyl
ethyl	isopropyl	p-carboxylphenyl-sulfonyl
ethyl	isopropyl	4-biphenylsulfonyl
ethyl	isopropyl	1-naphthylsulfonyl
ethyl	isopropyl	2-naphthylsulfonyl
ethyl	isopropyl	8-quinolinesulfonyl
ethyl	isopropyl	benzyl
ethyl	isopropyl	N-phenylcarbamoyl
ethyl	isopropyl	N-(p-butylphenyl)carbamoyl
ethyl	isopropyl	butylsulfonyl
ethyl	isopropyl	carbobenzyloxy
ethyl	isopropyl	methoxycarbonyl
ethyl	isopropyl	benzoyl
ethyl	isopropyl	methanesulfonyl
ethyl	isopropyl	phenylsulfonyl
ethyl	isopropyl	o-nitrophenylsulfonyl
ethyl	isopropyl	m-nitrophenylsulfonyl
ethyl	isopropyl	m-aminophenylsulfonyl
ethyl	R-2-butyl	m-methylphenylsulfonyl
ethyl	R-2-butyl	m-trifluoromethyl-phenylsulfonyl
ethyl	R-2-butyl	p-isopropylphenyl-sulfonyl
ethyl	R-2-butyl	p-propylphenylsulfonyl
ethyl	R-2-butyl	p-t-butylphenylsulfonyl
ethyl	R-2-butyl	p-carboxylphenyl-sulfonyl
ethyl	R-2-butyl	4-biphenylsulfonyl
ethyl	R-2-butyl	1-naphthylsulfonyl
ethyl	R-2-butyl	2-naphthylsulfonyl
ethyl	R-2-butyl	8-quinolinesulfonyl
ethyl	R-2-butyl	benzyl
ethyl	R-2-butyl	N-phenylcarbamoyl
ethyl	R-2-butyl	N-(p-butylphenyl)carbamoyl
ethyl	R-2-butyl	butylsulfonyl
ethyl	R-2-butyl	carbobenzyloxy
ethyl	R-2-butyl	methoxycarbonyl
ethyl	R-2-butyl	benzoyl
ethyl	R-2-butyl	methanesulfonyl
ethyl	R-2-butyl	phenylsulfonyl
ethyl	R-2-butyl	o-nitrophenylsulfonyl
ethyl	R-2-butyl	m-nitrophenylsulfonyl
ethyl	R-2-butyl	m-aminophenylsulfonyl
ethyl	S-2-butyl	m-methylphenylsulfonyl
ethyl	S-2-butyl	m-trifluoromethyl-phenylsulfonyl

R ¹	R ⁴	R ⁵
ethyl	S-2-butyl	p-isopropylphenyl-sulfonyl
ethyl	S-2-butyl	p-propylphenylsulfonyl
ethyl	S-2-butyl	p-t-butylphenylsulfonyl
ethyl	S-2-butyl	p-carboxylphenyl-sulfonyl
ethyl	S-2-butyl	4-biphenylsulfonyl
ethyl	S-2-butyl	1-naphthylsulfonyl
ethyl	S-2-butyl	2-naphthylsulfonyl
ethyl	S-2-butyl	8-quinolinesulfonyl
ethyl	S-2-butyl	benzyl
ethyl	S-2-butyl	N-phenylcarbamoyl
ethyl	S-2-butyl	N-(p-butylphenyl)carbamoyl
ethyl	S-2-butyl	butylsulfonyl
ethyl	S-2-butyl	carbobenzyloxy
ethyl	S-2-butyl	methoxycarbonyl
ethyl	S-2-butyl	benzoyl
ethyl	S-2-butyl	methanesulfonyl
ethyl	S-2-butyl	phenylsulfonyl
ethyl	S-2-butyl	o-nitrophenylsulfonyl
ethyl	S-2-butyl	m-nitrophenylsulfonyl
ethyl	S-2-butyl	m-aminophenylsulfonyl
propyl	ethyl	m-methylphenylsulfonyl
propyl	ethyl	m-trifluoromethyl-phenylsulfonyl
propyl	ethyl	p-isopropylphenyl-sulfonyl
propyl	ethyl	p-propylphenylsulfonyl
propyl	ethyl	p-t-butylphenylsulfonyl
propyl	ethyl	p-carboxylphenyl-sulfonyl
propyl	ethyl	4-biphenylsulfonyl
propyl	ethyl	1-naphthylsulfonyl
propyl	ethyl	2-naphthylsulfonyl
propyl	ethyl	8-quinolinesulfonyl
propyl	ethyl	benzyl
propyl	ethyl	N-phenylcarbamoyl
propyl	ethyl	N-(p-butylphenyl)carbamoyl
propyl	ethyl	butylsulfonyl
propyl	ethyl	carbobenzyloxy
propyl	ethyl	methoxycarbonyl
propyl	ethyl	benzoyl
propyl	ethyl	methanesulfonyl
propyl	ethyl	phenylsulfonyl
propyl	ethyl	o-nitrophenylsulfonyl
propyl	ethyl	m-nitrophenylsulfonyl
propyl	ethyl	m-aminophenylsulfonyl
propyl	propyl	m-methylphenylsulfonyl
propyl	propyl	m-trifluoromethyl-phenylsulfonyl
propyl	propyl	p-isopropylphenyl-sulfonyl

R ¹	R ⁴	R ⁵
propyl	propyl	p-propylphenylsulfonyl
propyl	propyl	p-t-butylphenylsulfonyl
propyl	propyl	p-carboxylphenyl-sulfonyl
propyl	propyl	4-biphenylsulfonyl
propyl	propyl	1-naphthylsulfonyl
propyl	propyl	2-naphthylsulfonyl
propyl	propyl	8-quinolinesulfonyl
propyl	propyl	benzyl
propyl	propyl	N-phenylcarbamoyl
propyl	propyl	N-(p-butylphenyl)carbamoyl
propyl	propyl	butylsulfonyl
propyl	propyl	carbobenzyloxy
propyl	propyl	methoxycarbonyl
propyl	propyl	benzoyl
propyl	propyl	methanesulfonyl
propyl	propyl	phenylsulfonyl
propyl	propyl	o-nitrophenylsulfonyl
propyl	propyl	m-nitrophenylsulfonyl
propyl	propyl	m-aminophenylsulfonyl
propyl	isopropyl	m-methylphenylsulfonyl
propyl	isopropyl	m-trifluoromethyl-phenylsulfonyl
propyl	isopropyl	p-isopropylphenyl-sulfonyl
propyl	isopropyl	p-propylphenylsulfonyl
propyl	isopropyl	p-t-butylphenylsulfonyl
propyl	isopropyl	p-carboxylphenyl-sulfonyl
propyl	isopropyl	4-biphenylsulfonyl
propyl	isopropyl	1-naphthylsulfonyl
propyl	isopropyl	2-naphthylsulfonyl
propyl	isopropyl	8-quinolinesulfonyl
propyl	isopropyl	benzyl
propyl	isopropyl	N-phenylcarbamoyl
propyl	isopropyl	N-(p-butylphenyl)carbamoyl
propyl	isopropyl	butylsulfonyl
propyl	isopropyl	carbobenzyloxy
propyl	isopropyl	methoxycarbonyl
propyl	isopropyl	benzoyl
propyl	isopropyl	methanesulfonyl
propyl	isopropyl	phenylsulfonyl
propyl	isopropyl	o-nitrophenylsulfonyl
propyl	isopropyl	m-nitrophenylsulfonyl
propyl	isopropyl	m-aminophenylsulfonyl
propyl	R-2-butyl	m-methylphenylsulfonyl
propyl	R-2-butyl	m-trifluoromethyl-phenylsulfonyl
propyl	R-2-butyl	p-isopropylphenyl-sulfonyl
propyl	R-2-butyl	p-propylphenylsulfonyl

R ¹	R ⁴	R ⁵
propyl	R-2-butyl	p-t-butylphenylsulfonfyl
propyl	R-2-butyl	p-carboxylphenyl-sulfonfyl
propyl	R-2-butyl	4-biphenylsulfonfyl
propyl	R-2-butyl	1-naphthylsulfonfyl
propyl	R-2-butyl	2-naphthylsulfonfyl
propyl	R-2-butyl	8-quinolinesulfonfyl
propyl	R-2-butyl	benzyl
propyl	R-2-butyl	N-phenylcarbamoyl
propyl	R-2-butyl	N-(p-butylphenyl)carbamoyl
propyl	R-2-butyl	butylsulfonfyl
propyl	R-2-butyl	carbobenzyloxy
propyl	R-2-butyl	methoxycarbonyl
propyl	R-2-butyl	benzoyl
propyl	R-2-butyl	methanesulfonfyl
propyl	R-2-butyl	phenylsulfonfyl
propyl	R-2-butyl	o-nitrophenylsulfonfyl
propyl	R-2-butyl	m-nitrophenylsulfonfyl
propyl	R-2-butyl	m-aminophenylsulfonfyl
propyl	S-2-butyl	m-methylphenylsulfonfyl
propyl	S-2-butyl	m-trifluoromethyl-phenylsulfonfyl
propyl	S-2-butyl	p-isopropylphenyl-sulfonfyl
propyl	S-2-butyl	p-propylphenylsulfonfyl
propyl	S-2-butyl	p-t-butylphenylsulfonfyl
propyl	S-2-butyl	p-carboxylphenyl-sulfonfyl
propyl	S-2-butyl	4-biphenylsulfonfyl
propyl	S-2-butyl	1-naphthylsulfonfyl
propyl	S-2-butyl	2-naphthylsulfonfyl
propyl	S-2-butyl	8-quinolinesulfonfyl
propyl	S-2-butyl	benzyl
propyl	S-2-butyl	N-phenylcarbamoyl
propyl	S-2-butyl	N-(p-butylphenyl)carbamoyl
propyl	S-2-butyl	butylsulfonfyl
propyl	S-2-butyl	carbobenzyloxy
propyl	S-2-butyl	methoxycarbonyl
propyl	S-2-butyl	benzoyl
propyl	S-2-butyl	methanesulfonfyl
propyl	S-2-butyl	phenylsulfonfyl
propyl	S-2-butyl	o-nitrophenylsulfonfyl
propyl	S-2-butyl	m-nitrophenylsulfonfyl
propyl	S-2-butyl	m-aminophenylsulfonfyl
allyl	ethyl	m-methylphenylsulfonfyl
allyl	ethyl	m-trifluoromethyl-phenylsulfonfyl
allyl	ethyl	p-isopropylphenyl-sulfonfyl
allyl	ethyl	p-propylphenylsulfonfyl
allyl	ethyl	p-t-butylphenylsulfonfyl

R ¹	R ⁴	R ⁵
allyl	ethyl	p-carboxylphenyl-sulfonyl
allyl	ethyl	4-biphenylsulfonyl
allyl	ethyl	1-naphthylsulfonyl
allyl	ethyl	2-naphthylsulfonyl
allyl	ethyl	8-quinolinesulfonyl
allyl	ethyl	benzyl
allyl	ethyl	N-phenylcarbamoyl
allyl	ethyl	N-(p-butylphenyl)carbamoyl
allyl	ethyl	butylsulfonyl
allyl	ethyl	carbobenzyloxy
allyl	ethyl	methoxycarbonyl
allyl	ethyl	benzoyl
allyl	ethyl	methanesulfonyl
allyl	ethyl	phenylsulfonyl
allyl	ethyl	o-nitrophenylsulfonyl
allyl	ethyl	m-nitrophenylsulfonyl
allyl	ethyl	m-aminophenylsulfonyl
allyl	propyl	m-methylphenylsulfonyl
allyl	propyl	m-trifluoromethyl-phenylsulfonyl
allyl	propyl	p-isopropylphenyl-sulfonyl
allyl	propyl	p-propylphenylsulfonyl
allyl	propyl	p-t-butylphenylsulfonyl
allyl	propyl	p-carboxylphenyl-sulfonyl
allyl	propyl	4-biphenylsulfonyl
allyl	propyl	1-naphthylsulfonyl
allyl	propyl	2-naphthylsulfonyl
allyl	propyl	8-quinolinesulfonyl
allyl	propyl	benzyl
allyl	propyl	N-phenylcarbamoyl
allyl	propyl	N-(p-butylphenyl)carbamoyl
allyl	propyl	butylsulfonyl
allyl	propyl	carbobenzyloxy
allyl	propyl	methoxycarbonyl
allyl	propyl	Benzoyl
allyl	propyl	methanesulfonyl
allyl	propyl	phenylsulfonyl
allyl	propyl	o-nitrophenylsulfonyl
allyl	propyl	m-nitrophenylsulfonyl
allyl	propyl	m-aminophenylsulfonyl
allyl	isopropyl	m-methylphenylsulfonyl
allyl	isopropyl	m-trifluoromethyl-phenylsulfonyl
allyl	isopropyl	p-isopropylphenyl-sulfonyl
allyl	isopropyl	p-propylphenylsulfonyl
allyl	isopropyl	p-t-butylphenylsulfonyl
allyl	isopropyl	p-carboxylphenyl-sulfonyl

R ¹	R ⁴	R ⁵
allyl	isopropyl	4-biphenylsulfonyl
allyl	isopropyl	1-naphthylsulfonyl
allyl	isopropyl	2-naphthylsulfonyl
allyl	isopropyl	8-quinolinesulfonyl
allyl	isopropyl	benzyl
allyl	isopropyl	N-phenylcarbamoyl
allyl	isopropyl	N-(p-butylphenyl)carbamoyl
allyl	isopropyl	butylsulfonyl
allyl	isopropyl	carbobenzyloxy
allyl	isopropyl	methoxycarbonyl
allyl	isopropyl	benzoyl
allyl	isopropyl	methanesulfonyl
allyl	isopropyl	phenylsulfonyl
allyl	isopropyl	o-nitrophenylsulfonyl
allyl	isopropyl	m-nitrophenylsulfonyl
allyl	isopropyl	m-aminophenylsulfonyl
allyl	R-2-butyl	m-methylphenylsulfonyl
allyl	R-2-butyl	m-trifluoromethyl-phenylsulfonyl
allyl	R-2-butyl	p-isopropylphenyl-sulfonyl
allyl	R-2-butyl	p-propylphenylsulfonyl
allyl	R-2-butyl	p-t-butylphenylsulfonyl
allyl	R-2-butyl	p-carboxylphenyl-sulfonyl
allyl	R-2-butyl	4-biphenylsulfonyl
allyl	R-2-butyl	1-naphthylsulfonyl
allyl	R-2-butyl	2-naphthylsulfonyl
allyl	R-2-butyl	8-quinolinesulfonyl
allyl	R-2-butyl	benzyl
allyl	R-2-butyl	N-phenylcarbamoyl
allyl	R-2-butyl	N-(p-butylphenyl)carbamoyl
allyl	R-2-butyl	butylsulfonyl
allyl	R-2-butyl	carbobenzyloxy
allyl	R-2-butyl	methoxycarbonyl
allyl	R-2-butyl	benzoyl
allyl	R-2-butyl	methanesulfonyl
allyl	R-2-butyl	phenylsulfonyl
allyl	R-2-butyl	o-nitrophenylsulfonyl
allyl	R-2-butyl	m-nitrophenylsulfonyl
allyl	R-2-butyl	m-aminophenylsulfonyl
allyl	S-2-butyl	m-methylphenylsulfonyl
allyl	S-2-butyl	m-trifluoromethyl-phenylsulfonyl
allyl	S-2-butyl	p-isopropylphenyl-sulfonyl
allyl	S-2-butyl	p-propylphenylsulfonyl
allyl	S-2-butyl	p-t-butylphenylsulfonyl
allyl	S-2-butyl	p-carboxylphenyl-sulfonyl
allyl	S-2-butyl	4-biphenylsulfonyl

R ¹	R ⁴	R ⁵
allyl	S-2-butyl	1-naphthylsulfonyl
allyl	S-2-butyl	2-naphthylsulfonyl
allyl	S-2-butyl	8-quinolinesulfonyl
allyl	S-2-butyl	benzyl
allyl	S-2-butyl	N-phenylcarbamoyl
allyl	S-2-butyl	N-(p-butylphenyl)carbamoyl
allyl	S-2-butyl	butylsulfonyl
allyl	S-2-butyl	carbobenzyloxy
allyl	S-2-butyl	methoxycarbonyl
allyl	S-2-butyl	benzoyl
allyl	S-2-butyl	methanesulfonyl
allyl	S-2-butyl	phenylsulfonyl
allyl	S-2-butyl	o-nitrophenylsulfonyl
allyl	S-2-butyl	m-nitrophenylsulfonyl
allyl	S-2-butyl	m-aminophenylsulfonyl
2,2-difluoroethyl	ethyl	m-methylphenylsulfonyl
2,2-difluoroethyl	ethyl	m-trifluoromethyl-phenylsulfonyl
2,2-difluoroethyl	ethyl	p-isopropylphenyl-sulfonyl
2,2-difluoroethyl	ethyl	p-propylphenylsulfonyl
2,2-difluoroethyl	ethyl	p-t-butylphenylsulfonyl
2,2-difluoroethyl	ethyl	p-carboxylphenyl-sulfonyl
2,2-difluoroethyl	ethyl	4-biphenylsulfonyl
2,2-difluoroethyl	ethyl	1-naphthylsulfonyl
2,2-difluoroethyl	ethyl	2-naphthylsulfonyl
2,2-difluoroethyl	ethyl	8-quinolinesulfonyl
2,2-difluoroethyl	ethyl	benzyl
2,2-difluoroethyl	ethyl	N-phenylcarbamoyl
2,2-difluoroethyl	ethyl	N-(p-butylphenyl)carbamoyl
2,2-difluoroethyl	ethyl	butylsulfonyl
2,2-difluoroethyl	ethyl	carbobenzyloxy
2,2-difluoroethyl	ethyl	methoxycarbonyl
2,2-difluoroethyl	ethyl	Benzoyl
2,2-difluoroethyl	ethyl	Methanesulfonyl
2,2-difluoroethyl	ethyl	Phenylsulfonyl
2,2-difluoroethyl	ethyl	o-nitrophenylsulfonyl
2,2-difluoroethyl	ethyl	m-nitrophenylsulfonyl
2,2-difluoroethyl	ethyl	m-aminophenylsulfonyl
2,2-difluoroethyl	propyl	m-methylphenylsulfonyl
2,2-difluoroethyl	propyl	m-trifluoromethyl-phenylsulfonyl
2,2-difluoroethyl	propyl	p-isopropylphenyl-sulfonyl
2,2-difluoroethyl	propyl	p-propylphenylsulfonyl
2,2-difluoroethyl	propyl	p-t-butylphenylsulfonyl
2,2-difluoroethyl	propyl	p-carboxylphenyl-sulfonyl
2,2-difluoroethyl	propyl	4-biphenylsulfonyl
2,2-difluoroethyl	propyl	1-naphthylsulfonyl

R ¹	R ⁴	R ⁵
2,2-difluoroethyl	propyl	2-naphthylsulfonyl
2,2-difluoroethyl	propyl	8-quinolinesulfonyl
2,2-difluoroethyl	propyl	benzyl
2,2-difluoroethyl	propyl	N-phenylcarbamoyl
2,2-difluoroethyl	propyl	N-(p-butylphenyl)carbamoyl
2,2-difluoroethyl	propyl	butylsulfonyl
2,2-difluoroethyl	propyl	carbobenzyloxy
2,2-difluoroethyl	propyl	methoxycarbonyl
2,2-difluoroethyl	propyl	benzoyl
2,2-difluoroethyl	propyl	methanesulfonyl
2,2-difluoroethyl	propyl	phenylsulfonyl
2,2-difluoroethyl	propyl	o-nitrophenylsulfonyl
2,2-difluoroethyl	propyl	m-nitrophenylsulfonyl
2,2-difluoroethyl	propyl	m-aminophenylsulfonyl
2,2-difluoroethyl	isopropyl	m-methylphenylsulfonyl
2,2-difluoroethyl	isopropyl	m-trifluoromethyl-phenylsulfonyl
2,2-difluoroethyl	isopropyl	p-isopropylphenyl-sulfonyl
2,2-difluoroethyl	isopropyl	p-propylphenylsulfonyl
2,2-difluoroethyl	isopropyl	p-t-butylphenylsulfonyl
2,2-difluoroethyl	isopropyl	p-carboxylphenyl-sulfonyl
2,2-difluoroethyl	isopropyl	4-biphenylsulfonyl
2,2-difluoroethyl	isopropyl	1-naphthylsulfonyl
2,2-difluoroethyl	isopropyl	2-naphthylsulfonyl
2,2-difluoroethyl	isopropyl	8-quinolinesulfonyl
2,2-difluoroethyl	isopropyl	benzyl
2,2-difluoroethyl	isopropyl	N-phenylcarbamoyl
2,2-difluoroethyl	isopropyl	N-(p-butylphenyl)carbamoyl
2,2-difluoroethyl	isopropyl	butylsulfonyl
2,2-difluoroethyl	isopropyl	carbobenzyloxy
2,2-difluoroethyl	isopropyl	methoxycarbonyl
2,2-difluoroethyl	isopropyl	benzoyl
2,2-difluoroethyl	isopropyl	methanesulfonyl
2,2-difluoroethyl	isopropyl	phenylsulfonyl
2,2-difluoroethyl	isopropyl	o-nitrophenylsulfonyl
2,2-difluoroethyl	isopropyl	m-nitrophenylsulfonyl
2,2-difluoroethyl	isopropyl	m-aminophenylsulfonyl
2,2-difluoroethyl	R-2-butyl	m-methylphenylsulfonyl
2,2-difluoroethyl	R-2-butyl	m-trifluoromethyl-phenylsulfonyl
2,2-difluoroethyl	R-2-butyl	p-isopropylphenyl-sulfonyl
2,2-difluoroethyl	R-2-butyl	p-propylphenylsulfonyl
2,2-difluoroethyl	R-2-butyl	p-t-butylphenylsulfonyl
2,2-difluoroethyl	R-2-butyl	p-carboxylphenyl-sulfonyl
2,2-difluoroethyl	R-2-butyl	4-biphenylsulfonyl
2,2-difluoroethyl	R-2-butyl	1-naphthylsulfonyl
2,2-difluoroethyl	R-2-butyl	2-naphthylsulfonyl

R ¹	R ⁴	R ⁵
2,2-difluoroethyl	R-2-butyl	8-quinolinesulfonyl
2,2-difluoroethyl	R-2-butyl	benzyl
2,2-difluoroethyl	R-2-butyl	N-phenylcarbamoyl
2,2-difluoroethyl	R-2-butyl	N-(p-butylphenyl)carbamoyl
2,2-difluoroethyl	R-2-butyl	butylsulfonyl
2,2-difluoroethyl	R-2-butyl	carbobenzyloxy
2,2-difluoroethyl	R-2-butyl	methoxycarbonyl
2,2-difluoroethyl	R-2-butyl	benzoyl
2,2-difluoroethyl	R-2-butyl	methanesulfonyl
2,2-difluoroethyl	R-2-butyl	phenylsulfonyl
2,2-difluoroethyl	R-2-butyl	o-nitrophenylsulfonyl
2,2-difluoroethyl	R-2-butyl	m-nitrophenylsulfonyl
2,2-difluoroethyl	R-2-butyl	m-aminophenylsulfonyl
2,2-difluoroethyl	S-2-butyl	m-methylphenylsulfonyl
2,2-difluoroethyl	S-2-butyl	m-trifluoromethyl-phenylsulfonyl
2,2-difluoroethyl	S-2-butyl	p-isopropylphenyl-sulfonyl
2,2-difluoroethyl	S-2-butyl	p-propylphenylsulfonyl
2,2-difluoroethyl	S-2-butyl	p-t-butylphenylsulfonyl
2,2-difluoroethyl	S-2-butyl	p-carboxylphenyl-sulfonyl
2,2-difluoroethyl	S-2-butyl	4-biphenylsulfonyl
2,2-difluoroethyl	S-2-butyl	1-naphthylsulfonyl
2,2-difluoroethyl	S-2-butyl	2-naphthylsulfonyl
2,2-difluoroethyl	S-2-butyl	8-quinolinesulfonyl
2,2-difluoroethyl	S-2-butyl	benzyl
2,2-difluoroethyl	S-2-butyl	N-phenylcarbamoyl
2,2-difluoroethyl	S-2-butyl	N-(p-butylphenyl)carbamoyl
2,2-difluoroethyl	S-2-butyl	Butylsulfonyl
2,2-difluoroethyl	S-2-butyl	Carbobenzyloxy
2,2-difluoroethyl	S-2-butyl	methoxycarbonyl
2,2-difluoroethyl	S-2-butyl	benzoyl
2,2-difluoroethyl	S-2-butyl	methanesulfonyl
2,2-difluoroethyl	S-2-butyl	phenylsulfonyl
2,2-difluoroethyl	S-2-butyl	o-nitrophenylsulfonyl
2,2-difluoroethyl	S-2-butyl	m-nitrophenylsulfonyl
2,2-difluoroethyl	S-2-butyl	m-aminophenylsulfonyl

UTILITY

The compounds of the present invention are expected to inhibit the activity of 26S proteasome. The 26S proteasome inhibition is demonstrated using assays for proteasome activity, for example, using the assay described below for assaying inhibitors of proteasome. The compounds of Formula (I) are expected to show activity against the proteasome in cells, as demonstrated by the cellular assay described

below. Thus, the compounds of Formula (I) are potentially useful in the treatment of cancer, muscle-wasting syndromes, inflammation and immune response-mediated conditions.

5 Partial Purification of Mammalian Proteasomes

Mammalian proteasomes were partially purified based on a published protocol (Vinitsky, A. et al, J. Immunol. 159:554 (1997)). Fractions enriched in proteasomal activity were isolated from HL60 human promyelocytic leukemia cells grown in RPMI media under normal tissue culture conditions. Cells were pelleted by centrifugation, rinsed with PBS (20 mM sodium phosphate, pH 7.4, 140 mM NaCl) solution and repelleted by centrifugation. Cell pellets were stored at -80°C . The cell pellet was thawed and resuspended in tris-HCl buffer (50 mM, pH 7.5). The suspension was homogenized in a dounce homogenizer (30 strokes) and centrifuged at 100,000g for 30 min at 4°C . Polyethylene glycol was added to the supernatant to make a 5% solution. The mixture was centrifuged at 15,000g for 30 minutes at 4°C . The pellet was discarded and polyethylene glycol was added to the supernatant to make the final concentration of 12% v/v. The mixture was centrifuged again at 15,000g for 30 minutes at 4°C . The proteasome-enriched supernatant was stored at -80°C and diluted immediately prior to use in enzyme assay buffer.

20

Enzyme Assays

The proteolytic activity of the proteasome was determined by utilizing synthetic peptide substrates specific for the chymotrypsin-like activity of the proteasome. The activity of the proteasome towards the cleavage of succinyl-Leu-Leu-Val-Tyr-(7-amino-4-methyl coumarin) was measured by the increased fluorescence of the coumarin moiety after cleavage from the peptide. The sources of proteasomes were the proteasome enriched fraction from HL60 cells described above and pure commercially available 20S human proteasomes (Affiniti Research Products, Mamhead, UK). The proteasome enriched fraction was assayed at 50 μ g/ml concentration in a buffer containing 150 mM tris-EDTA (pH 7.4). The pure 20S proteasomes were assayed at 3 μ g/ml concentration in a buffer containing 25 mM hepes, 0.5 mM EDTA, 0.03% sodium dodecyl sulphate (pH 7.6). The activities of

both preparations were monitored over time by measuring the increase in fluorescent signal (excitation at 360 nm; emission at 460 nm) in a Cytofluor Series 4000 multiwell platereader (Applied Biosystems).

5

Inhibitor Evaluation *In vitro*

Compounds were evaluated for proteasome inhibition under steady state conditions where substrate concentration is $\leq K_m$ and the amount of substrate hydrolyzed during the reaction is less than 10% of the total substrate in the reaction. Appropriately diluted proteasome preparations were incubated with the inhibitor for 10 30 minutes at 37°C prior to the initiation of the reaction by the addition of substrate. The substrate concentration for each assay was at or below the apparent K_m levels determined for each preparation (pure 20S proteasome, 10 μM ; proteasome-enriched fraction, 50 μM). The reactions proceeded for 60 minutes at 37°C before reading in the fluorescent platereader. Inhibition was quantitated by determination of the IC_{50} 15 values for the given conditions. A number of compounds from this invention were tested and were active with IC_{50} values ranging from low nM to 100 μM .

Inhibitor Evaluation in Cellular Assays.

Two methods were used to evaluate the compounds of this invention in 20 cellular assays. The first method used to evaluate the inhibitory action of test compounds on the proteasome in intact cells is based on the fact that this protein complex is a key component of the ubiquitin-proteasome pathway of protein degradation. The proteasome catalyzes the degradation of intracellular ubiquitin-protein conjugates. Accordingly, treatment of cultured tumor cells with proteasome 25 inhibitors can result in increased levels of ubiquitin-protein conjugates. The following method was used to detect proteasome inhibition in cultured cells. Cultured tumor cells such as LX-1 human lung carcinoma cells were plated in RPMI media with 10% serum containing antibiotics and placed in a standard tissue culture incubator at 37°C and 5% CO_2 . One day after plating the cells, various concentrations 30 of test compounds were added. After 4-24h of exposure to the compounds, the plates were rinsed with PBS and then lysed on the plates by direct addition of SDS buffer: 50 mM tris, pH 7.4 and 1% SDS containing a standard protease inhibitor cocktail.

The lysate was centrifuged at 10,000g for 20 minutes. The supernatant was assayed for protein content with a standard protein determination assay. The samples were loaded onto a standard SDS-PAGE gel and the proteins were separated by electrophoresis. The gel contents were then electroblotted onto PVDF filters and ubiquitin conjugates were detected with anti-ubiquitin antibodies (Dako, USA). A number of compounds from this invention were tested in this cellular assay and produced significant increases in the levels of cellular ubiquitin conjugates at concentrations ranging from 0.5 to 50 μ M.

The second cellular assay used to evaluate the compounds of this invention was based on the fact that proteasome inhibitors have been shown to decrease cancer cell proliferation both *in vitro* and *in vivo* (Adams, J. 1999 Cancer Res. 59, 2615). A number of compounds from this invention were tested in a standard assay to detect inhibition of proliferation of cultured tumor cells. For example, LX-1 cells were plated in RPMI media containing 10% serum in multiwell plates. Approximately 24h after plating, various concentrations of the test compounds were added to the cells. After an exposure period of 48-72h, survival was determined by adding a common tetrazolium compound that was converted by cellular mitochondria into a formazan compound. The formazan compound absorbs light at 490 nm and was quantified using a visible light platereader. The IC₅₀ values for cytotoxicity were calculated from the dose response curves. Various compounds from this invention were tested, resulting in a range of IC₅₀ values from <1 μ M to 100 μ M.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations. Various equivalents, changes and modifications may be made without departing from the spirit and scope of this invention, and it is understood that such equivalent embodiments are part of this invention.